EVALUATING THE SEASONAL CHANGES IN CALCIUM CONCENTRATION AND DISTRIBUTION IN APPLE FRUIT AFTER APPLICATION OF DIFFFERENT CALCIUM FERTILISATION STRATEGIES

By

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DECLARATION

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SUMMARY

Localized calcium (Ca) deficiencies frequently develop independent from total Ca supply and total fruit Ca concentration. Bulk mineral analyses is therefore not completely suitable for determination of the susceptibility of fruit in developing Ca-linked fruit physiological disorders like bitter pit, as it supplies insufficient information regarding the spatial accumulation of Ca within the fruit. Additional Ca is applied either as soil or foliar applications, where soil applications are applied either after fruit set (pre-harvest) or in the period after harvest. The contribution of these different methods of Ca application to the total Ca concentration in 'Braeburn' fruit was quantified over three consecutive growing seasons. Foliar applications have been proven to be successful in suppressing bitter pit development and improving the Ca status of the fruit. The effectiveness of different formulations of foliar Ca products in influencing these parameters was also determined. Furthermore, the distribution pattern of fruit Ca resulting from different application strategies was mapped using particle induced X-ray emission technology.

In the 'Braeburn' trial, mineral analyses indicated no significant differences between treatments in terms of Ca concentration at 80 days after full bloom (dafb). However, when soil applications occurred with active root growth (visually monitored), treatments differed significantly 80 dafb. Weekly foliar Ca applications from 28 dafb resulted in higher fruit Ca concentrations 80 dafb than a pre-harvest soil Ca application (January, 2010). A possible explanation for the inefficiency of pre-harvest soil Ca is the disintegration of xylem vessels from 40 dafb (before root uptake) for sensitive cultivars such as Braeburn. Bitter pit initiation has been shown to occur in the earlier part of the season. This, together with a reduced Ca supply to the fruit early in the season due to xylem disintegration (for sensitive cultivars), indicates the importance of early season foliar Ca applications.

PIXE analyses were used to establish the radial Ca distribution in apples. Ca was concentrated in the skin and core, with very low values in the outer cortex. PIXE results indicated fruit Ca concentrations to be the lowest in the pre-harvest soil application treatment. This was in agreement with mineral analyses results. Ca enriched areas resulting from effective Ca delivery via the vascular bundles, had a profound effect on fruit Ca concentrations in the immediate core and cortex. At harvest, this effect was much more subtle and emphasizes the importance of untimely xylem rupturing on eventual fruit Ca concentration. At 80 dafb, treatments where foliar Ca was applied showed higher Ca concentrations in the outer cortex (where symptoms of bitter pit typically occur).

Calflo™ fruit had significantly higher Ca concentrations in 'Braeburn' compared to fruits from Foliar GS™ and GG™ treatments. Calflo™ and Calcimax™ had a higher active Ca percentage (12%) compared to Foliar GS™M and GG™ (10%). Adding the Lecithin™ (surfactant) to Calcimax™ is not recommended as it did not improve its uptake.

In 'Golden Delicious', the commercial spray program of seven, weekly foliar applications (Calcinit™) resulted in fruit with significantly higher Ca concentrations compared to other treatments.

OPSOMMING

Kalsium (Ca) tekorte ontstaan gewoonlik in gelokaliseerde areas in die appelvrug en ontwikkel dikwels ten spyte van voldoende totale vrug Ca. Minerale analises van heel vrugte verskaf dus nie genoeg inligting aangaande die verspreiding van Ca in die vrug wanneer die ontwikkelling van fisiologiese defekte soos bitterpit ter sprake is nie. Addisionele Ca word gewoonlik aangewend as blaar- of grondtoedienings, waar grondtoedienings tipies voor-oes (net na set) of in die na-oes periode, toegedien word. Die bydraes van die verskillende toedieningsmetodes tot die totale Ca konsentrasie van 'Braeburn' appels is geëvalueer oor drie agtereenvolgende seisoene. Blaartoedienings van Ca word algemeen gebruik om die voorkoms van bitterpit te beheer en die Ca konsentrasie van die vrug te verhoog. Die effektiwiteit van `n reeks blaartoedienings-produkte om hierdie faktore te verbeter, is ook ondersoek. Die spesifieke verspreiding van die Ca in die vrug is gekarteer na gelang van elke toediening deur middel van PIXE-analises (Particle induced X-ray emission).

In die 'Braeburn' proef was daar geen beduidende verskille in terme van vrug Ca konsentrasie op 80 dnvb (dae na volblom) nie. Daarteenoor, was daar wel beduidende verskille by 80 dnvb toe grond toedienings saam met aktiewe wortelgroei geskied het (visuele inspeksie). Weeklikse blaartoedienings vanaf 21 dnvb het gelei tot vrugte met betekenisvol hoër Ca konsentrasies as die behandeling waar grondtoedienings slegs voor-oes geskied het (Januarie 2010). `n Moontlike oorsaak vir die oneffektiwiteit van voor-oes grondtoedings is die vroeë disintigrasie van xileem vesels in die vrug (soms voor 40 dnvb en voor die aanvang van wortelopname) in sensitiewe kultivars soos 'Braeburn'. Hierdie vroeë inhibering van Ca voorsiening, tesame met die vroeë inisiasie van bitterpit, beklemtoon die belangrikheid van blaarbespuitings vroeg in die seisoen.

Die PIXE-analises wat aangewend is om die radiale verspreiding van Ca in die vrug te bepaal het getoon dat Ca meestal in die skil en kern van die vrug gekonsentreer was, met baie lae konsentrasies in die buitenste korteks. Die laagste Ca konsentrasies is waargeneem in vrugte van die behandeling waar voor-oes Ca slegs as `n grondtoediening geskied het. Hierdie waarneming is in ooreenstemming met die mineraalanalise resultate. Ca verykte areas, afkomstig van die naby geleë vaatbundels (xileem vesels), het egter die grootste effek op vrug Ca konsentrasie gehad. Hierdie effek was nie so groot by oes nie en beklemtoon dus die belangrikheid van die funksionaliteit van die vaatbundels. Blaartoedienings kon die Ca konsentrasie in die buitenste korteks suksesvol verhoog - waar simptome van bitterpit tipies voorkom.

Die Calflo™ behandeling het beduidende hoër Ca konsentrasies gehad as die Foliar GS™ en GG™ behandelings. Die Calflo™ en Calcimax™ behandelings het `n hoër aktiewe Ca persentasie (12%) relatief tot die Foliar GS™ en GG™ (10%) behandelings bevat. Die byvoeging van Lecithin™ by Calcimax™ word nie aanbeveel nie, omdat dit geensins Ca opname vermeerder het nie.

In die ‘Golden Delicious’ proef het die kommersiële behandeling (Sewe weeklikse spuite van Calcinit™) gelei tot vrugte met die hoogste Ca konsentrasie van al die behandelings.

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TABLE OF CONTENTS

This thesis presents a compilation of manuscripts where each chapter is an individual entity and some repetitions between chapters, therefore, have been unavoidable. The different styles used in this thesis are in accordance with the agreements of different journals used for submission of manuscripts from the thesis. Chapters 1, 2 and 4 were written for the Journal of Horticultural Science and Biotechnology, while Chapter 3 was written for Scientia Horticulturae.

General Introduction

Calcium (Ca) deficiencies occur in a wide variety of apple orchards across the globe. Symptoms often occur in spite of sufficient amounts of Ca in the tree and fruit (Saure, 2005). Commercially, external Ca is supplied in the form of either soil applications after fruit set and after harvest, or as a series of foliar sprays. Most of the Ca is transported toward the leaves after absorption form the soil (Bangerth, 1979), while an insufficient distribution of the Ca contained within the fruit result in localized deficiencies (Saure, 2005).

The aim of this study was to firstly determine how different Ca application strategies would influence the quality and total Ca concentration of the fruit. Active root growth is essential for absorption of Ca from the soil (Bangerth, 1979), because Ca can only enter the root system at newly developed root tips (Himelrick and McDuffie, 1983). The timing of root flushes typically vary between localities (Eissenstat *et al*., 2006) and this explains in part why soil Ca is ineffective if not applied at the time of active root growth (Bangerth, 1979).

Ca is almost exclusively transported in the xylem vessels (Himelrick and McDuffie, 1983). These vessels are very rigid and eventually rupture within the expanding fruit (Drazeta *et al*., 2004). Disintegration of the xylem occurs from as early as 45 days after full bloom (dafb) in some cultivars and this has considerable implications with regard to Ca transport from the roots and tree reserves (Drazeta *et al*., 2004, Neilsen *et al*., 2005).

Apples with low Ca concentrations are prone to developing bitter pit, a physiological disorder that influences the general appearance of the fruit and is commonly accompanied by substantial economic losses (Rousseau, 1972; Ferguson and Watkins, 1983; Saure, 2005). Most of the Ca in apple fruit is concentrated in the skin and core, with considerably lower concentrations in the outer cortex (Ferguson and Watkins, 1983; Himelrick and McDuffie, 1983). The tissues just beneath the peel will benefit most from Ca sprays (Hanekom, 1975), due to the confinement of movement further into the cortex. As no significant remobilization of Ca takes place from the leaves, only the Ca that penetrates the fruit surface directly will contribute towards the fruit Ca status (Hanekom, 1975; Saure 2002; Schlegel and Schönherr, 2002; Saure, 2005). Ca foliar applications, both globally and in South Africa, have been used successfully as routine procedure to control and reduce the incidence of bitter pit (De Villiers and Hanekom, 1977; Terblanche *et al*., 1980; Ferguson and Watkins, 1989; Saure 2002; 2005; Lötze and Theron, 2007).

Early season foliar sprays mainly penetrate the surface of the fruit through trichomes and stomata (Schlegel and Schönherr, 2002; Lötze and Theron, 2006), while penetration occurs through open lenticels and cracks when applied at a later stage (Harker and Ferguson, 1991; Trentham *et al*., 2008). Although a considerably higher amount of Ca enters the fruit with late season sprays (Harker and Ferguson, 1991), the variable permeability of the lenticels result in a less uniform distribution of Ca in the outer cortex and this may provide a possible explanation for the disorderly assortment of bitter pit lesions (Schlegel and Schönherr, 2002). The rate of penetration is much higher when foliar Ca is applied before 45 dafb (Schlegel and Schönherr, 2002; Lötze and Theron, 2006). Although smaller amounts of Ca penetrate the fruit due to a

smaller fruit surface, early season Ca sprays have been proven more effective in reducing bitter pit in some instances (Neilsen and Neilsen, 2002; Schlegel and Schönherr, 2002; Neilsen *et al*., 2005; Lötze and Theron, 2007; Lötze *et al*., 2008).

The second aim of the project was to evaluate the efficiency of local foliar Ca formulations on fruit Ca concentration. The specific formulation of a product has a considerable influence on its penetration efficiency (Schlegel and Schönherr, 2002). Joubert (2007) has shown how foliar products with different amounts of active Ca and applied at different frequencies, have variable effects on fruit Ca concentration and bitter pit incidence. The effect of product formulation and timing of application on fruit Ca concentration and quality was also investigated.

Lastly, the distribution pattern of Ca when applied externally (soil versus foliar applications), was quantified using Particle induced X-ray emission (PIXE) analyses. Previously, PIXE has been proven to be a successful method in determining the elemental distribution of apples with regard to bitter pit after storage (Meyer *et al*., 1982).

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Chapter 1: Literature Review

The uptake and translocation of calcium in the apple tree with regard to bitter pit incidence and fruit quality

1. Introduction

Calcium (Ca) deficiencies and related physiological disorders are a common occurrence in apple orchards throughout the world, with huge economic losses occurring to date. These deficiencies are not easily alleviated, as symptoms can also occur in orchards with adequate Ca supply (Saure, 2005). Aside from Ca deficient soils, there are numerous problems with the uptake and translocation of this element with regard to the fruit (Rousseau, 1972; Hanekom, 1975; Himelrick and McDuffie, 1983; White, 2001; Drazeta *et al*., 2004; Saure, 2005; Eissenstat *et al.*, 2006). Marschner (1995) also showed that leaf Ca concentrations increase with Ca application to the soil (roots), but this does not necessarily occur in fruit after (Saure, 2005). A possible explanation is that plants have developed natural ways to reduce the Ca content in the fruit in order to maintain rapid fruit growth (Saure, 2005). Thus, although a significant number of publications are available on Ca involvement in fruit quality, Ca applications to apple orchards to reduce physiological disorders like bitter pit, have still not achieved complete control under field conditions.

A holistic approach is necessary to manipulate an increase Ca concentration in the fruit as well as a uniform distribution of Ca in the fruit tissues. It is important to understand how calcium is absorbed from the soil and distributed through the tree structures in order to establish a possible strategy to reduce related disorders. Thus, this review attempts to utilise existing

literature to re-construct the pathway of Ca uptake and translocation in the apple tree and add the latest findings, in an attempt to increase the efficiency of applied Ca for improved fruit quality.

1.1 Implications of low fruit Ca concentrations and the importance of foliar Ca applications

1.1.1 Basic functions of Ca in the fruit

Ca is an essential element that performs a wide range of metabolic functions in fruit. It plays an integral role in retaining the proper functioning and structure of the cell membrane (Rousseau, 1972; Zocchi and Mignani 1995). Ca further stabilizes cell wall structures and ensures good cell to cell adhesion by the binding action of negative cell surfaces (Zocchi and Mignani, 1995). It also fulfils a structural role in the middle lamellae between adjacent cells and plays an important role in cell division (Zocchi and Mignani, 1995).

1.1.2 Physiological disorders in apple fruit

Bitter pit is a physiological disorder that often occurs in apple fruit (Bangeth, 1979; Terblanche *et al*., 1980; Ferguson and Watkins, 1983; 1989; Saure, 2005; Lötze and Theron, 2006; 2007; 2008). It is characterized by dry areas of corky cells just beneath the skin and causes suppressed areas on the fruit surface (Rousseau, 1972; Hanekom, 1975). These symptoms are always more pronounced toward the calyx end of the fruit due to the natural Ca gradient, as mentioned above.

Localized deficiencies in the outer cortex increase the permeability of the cell membranes, resulting in solutes that leak out of cells structures (Rousseau, 1972). Solute leakage leads to a total collapse of the cell wall and a disintegration of the tonoplast, leading to a reduction in compartmentalization of protoplasmic enzymes and substrates within the cell (Rousseau, 1972). This eventually results in the total disruption and even death of whole cells (Rousseau, 1972). Thus, problems with marketing of fruit typically occur because of the negative effects of these symptoms on the visual aspects of the fruit, resulting in substantial economic losses (Rousseau, 1972; Hanekom, 1975).

Symptoms of bitter pit typically become more pronounced during extended periods of cold storage, and fruit that seem unaffected at the time of harvest, tend to develop lesions before reaching overseas markets (Rousseau, 1972; Hanekom, 1975). As ripening of the fruit progresses through storage, ethylene gas is produced and this increases the rate of respiration and ripening of the fruit (Pons, 2008). Ca tends to move into the cytoplasm where these metabolic reactions occur, resulting in a decrease of levels in the cell wall and membrane (Pons, 2008). Foliar Ca applications have been reported to be effective in delaying respiration through storage, thus reducing further development of bitter pit (Saure, 2005). Furthermore, the role that Ca plays in stabilizing the cell wall structure while retaining membrane integrity and ensuring the proper turgidity of cells, is important in preserving the desired textures of the fruits, whereby maintaining fruit firmness throughout storage (Bangerth, 1979; Clarkson, 1984; Siddiqui and Bangerth, 1995; Zocchi and Mignani 1995).

1.2 Soil: Ca uptake, translocation and accumulation

1.2.1 Ca uptake from the soil

Calcium moves from the soil solution to the root surface mainly through mass flow, where it is then absorbed by the numerous root hairs contained on the root surface (Himelrick and McDuffie, 1983). After this initial uptake, there are two main mechanisms of transport from the root surface towards the vascular tissue of the plant: i) Ca can move via the apoplast through intercellular spaces and cell walls or, ii) Ca transport occurs via the symplast and cytoplasm (White, 2001). Symplastic transport occurs from cell to cell, linked by middle lamellae. The apoplastic movement of Ca, within the roots, is the quickest and most efficient way of uptake and deposition into the xylem, but this movement can be restricted by the existence of the suberised casparian bands. These develop around a layer of endodermis cells that surrounds the stele (White, 2001). For Ca-ions to be transported across the restrictive endodermis layer, ions need to move into the cytoplasm of these cells via ion channels (White, 2001). Ca concentrations in the symplast are maintained at very low levels, about a thousand times lower than the fraction transported apoplasticaly (Saure, 2005). Therefore, symplastic Ca transport is not sufficient in supplying Ca to developing shoots and fruits (White, 2001; Saure, 2005).

In actively growing root tips, the casparian bands that surround the stele, are poorly or un-developed (un-suberized) (White, 2001). Ca ions can be transported at much higher rates by moving directly in the vascular tissue, via the apoplast, in un-suberized root tissue. Thus, most of the Ca will be absorbed through these regions at the root apex (White, 2001). It is therefore apparent that suboptimal soil conditions can pose huge threats in terms of Ca uptake, as active

root growth is essential for this purpose (Eissenstat *et al*., 2006). Furthermore, there are only two periods of active root growth in apple trees, early spring and after harvest (Northern Hemisphere). These periods typically vary between seasons and localities and play a critical role in determining the timing of Ca fertilization (Eissenstat *et al.,* 2006). Adverse soil conditions like high salinity, water logging, drought, nutrient deficiency and soil compaction, as well as root diseases, will affect root growth and subsequent absorption of nutrients, especially in the case of Ca. Antagonistic ions like potassium (K), sodium (Na), aluminum (Al) and magnesium (Mg), and a low soil pH can further lead to reduced Ca absorption (Warner, 2008; Himelrick and McDuffie, 1983).

1.2.2 Ca transport and regulation

Ca is highly immobile in the phloem and will be transported upward in the plant almost exclusively via the xylem (Himelrick and McDuffie, 1983). Xylem vessels stretch through the pedicel of the fruit where the vascular bundles eventually form an interconnected network of veins in the fruit flesh.

As opposed to K, which flows relatively easily in the xylem, the divalent Ca ion becomes strongly adsorbed to the negative inner surfaces of the vessels (Tromp, 1979). Upward movement of Ca in the xylem occurs not only through mass flow, but also by ion exchange and this undoubtedly slows down transport (Tromp, 1979). Ca ions must first saturate all exchange sites in the lower regions of each vessel, before it is to be transported further towards developing fruits and leaves (Saure, 2005).

About 50% of the Ca transported in the xylem is in an ionic form. The rest of the Ca exists as complexes with organic acids such as citrate and malic acid (Bradfield, 1975). These acids can considerably increase the mobility of Ca as it forms neutral or negatively charged complexes and reduce the degree to which Ca is adsorbed to the negatively charged surfaces of the xylem vessels (Bradfield, 1975). The mobility of Ca can thus be significantly improved if the presence of these acids is increased within the tree.

Fruits receive water primarily from the xylem in the beginning of the season. At this time, Ca freely enters the fruit via the pedicel and Ca is distributed throughout the whole fruit. As the season progresses, tree foliage increases and the water supply shifts from a net xylem to phloem dominated transport (Saure, 2005). Ca import via the xylem becomes largely restricted, while photosynthetic assimilates are readily transported from the leaves, resulting in an insufficient Ca supply to the rapidly expanding and metabolically accelerating fruit (Himelrick and McDuffie, 1983; Saure, 2005).

As the fruitlet develops, there is an increasing competition for Ca between sinks (Bangerth, 1979). The developing leaf canopy has a much higher transpiration rate (sink strength) when compared to the fruits (Bangerth, 1979). Translocation of water and Ca will increasingly be adjusted towards developing shoots and leaves, thus away from the developing fruit (Bangerth, 1979; Clarckson, 1984). Over application of nitrogen will increase vegetative growth - resulting in a further decrease in Ca transported towards the developing fruits, accompanied by a possible reduction in of crop load (Saure, 2005). This also coincides with generally bigger fruit and additional dilution of Ca because of excess sap that is stored (Saure, 2005).

The pedicel serves as a natural bottleneck for xylem flow into the fruit (Drazeta *et al*., 2004). Hydraulic conductance in the xylem is directly related to the cross sectional area of its vessels, resulting in the bigger vessels being responsible for most of the Ca translocation. Low rates of Ca transport and sap conductance occurs through the pedicel because of small vessels contained in this area (Drazeta *et al*., 2004). It has also been proposed that Ca flow can be forced out of the xylem into the phloem at these critical branch points and can thus reduce Ca transport into the fruit (Lee, 1989; Düring and Lang, 1993; Drazeta *et al*., 2004).

Xylem vessels comprise very rigid, dead cells that tend to rupture and become dysfunctional as the fruit expands (Drazeta *et al*., 2004). Water transport will therefore be interrupted and Ca transport will cease at the broken ends (Drazeta *et al*., 2004). These findings are in agreement with Ford and Quinlan (1979), explaining the blockage of Ca at the pedicel when applied to the roots in midsummer. Cultivars are more susceptible to bitter pit and Ca deficiency where early xylem breakage occurs (Drazeta *et al*., 2004). This occurs much earlier in the season for cultivars such as ‘Braeburn’ (more susceptible to bitter pit) compared to ‘Granny Smith’.

1.2.3 Remobilization from Ca reserves

At the beginning of the growing season, large amounts of nutrients are remobilized from reserve tissues in the tree towards developing shoots and flowers. Remobilisation from the reserves supplies developing shoots with Ca before root uptake has started (Ferguson, 1980). This was illustrated via an increase in the concentrations of K, Mg, P and Ca in shoot sap just before emergence of the first leaves in kiwi fruit (Ferguson, 1980). Ca concentrations reached a

sharp peak in the xylem sap about one week after bud break and this early remobilization can supply 25% of the Ca contained in the new growth in apples (Bradfield, 1976; Terblanche *et al*., 1979a; Tromp, 1979; Himelrick and McDuffie, 1983).

About 40% of the Ca in an apple shoot is located in the bark, with smaller amounts occurring in the wood (Ferguson and Turner, 1981). About 50% of this Ca becomes fixed as Ca oxalate and will not be remobilized to the fruit in the short term (Ferguson and Turner, 1981). Most of the Ca in new growth will therefore derive from remobilized Ca in smaller amounts from reserve Ca in the wood (Ferguson and Turner, 1981). Some of the free Ca stored in the bark can be remobilized towards the wood in the long term, however Ca associated with the bark will not be transported directly to the new growth (Ferguson and Turner, 1981).

1.2.4 Accumulation of Ca in the developing fruit

Ca differs from most of the other elements because large amounts are transported to developing shoots and leaves compared to fruit (Saure, 2005). Around fruit set, fruit Ca concentrations will be the highest, and afterwards will decline as the season progresses because of the dilution effect of expanding fruit (Saure, 2005). Bigger fruit will therefore have a lower Ca concentration compared to smaller fruit (Saure, 2005).

During the first phase of fruit growth, a rapid uptake of Ca occurs during active cell division (Wilkinson, 1968). Most of the Ca transported from the roots will be accumulated in this early stage of fruit growth, resulting in a uniform distribution of Ca in the fruit cortex (Saure, 2005). Transport of Ca to the fruit tends to decrease as the xylem dominated influx gradually changes to phloem import (Zocchi & Mignani 1995; Saure, 2005). As mentioned before, uptake

will eventually stop completely when xylem vessels rupture later in the season (Drazeta *et al*., 2004).

At harvest, Ca concentrations will be the highest in the skin and core and the lowest, in the tissues in the outer cortex (Himelrick and McDuffie, 1983). There will also be a natural lateral decline in the Ca concentration from the stem toward the calyx end of the fruit (Wilkinson and Perring, 1961; 1964; Terblanche, 1976; Terblanche et al., 1979a; Himelrick and McDuffie, 1983; Saure, 2005). These differences in Ca concentration can be attributed to a higher growth rate of the fruit flesh in the outer cortex and calyx, accompanied with a natural lack in distribution of vascular bundles in these areas (Hanekom, 1975; Saure, 2005). Deficiency symptoms generally develop if Ca concentrations are below 700 ppm in the skin and 200 ppm in the cortex (Hanekom, 1975; Himelrick and McDuffie, 1983). A total fruit Ca concentration, above 4.5 mg.100g FW^{-1} at harvest is regarded as sufficient for good fruit quality (Terblanche, 1985).

1.3 Penetration and uptake of foliar Ca from the fruit surface

Previous studies have shown that the application of pre harvest Ca sprays significantly stabilizes cell membranes, thereby reducing the permeability of these cells and the accompanied leakage of solutes (Rousseau, 1972; Hanekom, 1975). Foliar applications have also been successful as a preventative measure in order to control bitter pit both in South Africa and internationally (De Villiers and Hanekom, 1977; Terblanche *et al*., 1980; Ferguson and Watkins, 1989; Saure 2002; 2005; Lötze and Theron, 2007). A 16% reduction in the incidence of bitter pit was achieved by Terblanche *et al*. (1980) with the application of late season Ca sprays on

'Golden Delicious'. Lötze *et al*. (2008) showed that bitter pit can be controlled effectively with the application of a series of six early season foliar sprays under South African conditions. Thus, foliar applications can be viewed as a possible application method to increase fruit Ca. The following factors influence the efficiency of the application:

1.3.1 Anatomy

The fraction of foliar Ca that is absorbed by the leaves will not contribute significantly towards the fruit Ca status. Therefore, in terms of alleviating deficiencies, it is important that Ca is directly applied to the fruit surface (Hanekom, 1975; Schlegel and Schönherr, 2002; Saure, 2005).

The fruit cuticle and its overlaying waxy layer serves as the main restriction with regard to penetration of foliar applied Ca (Harker and Ferguson, 1991). There are small portions of Ca that can penetrate through the skin, but these amounts usually do not contribute significantly to the Ca nutrient status of the fruit (Harker and Ferguson, 1991).

1.3.2 Timing of application

Foliar sprays applied early in the season, before 45 days after full bloom (dafb), mainly penetrates the fruit through trichomes and stomata on the fruit surface (Schlegel and Schönherr, 2002). After 45 dafb, open lenticels and cracks in the cuticle and outer waxy layer serves as penetration pathway of foliar applied Ca (Harker and Ferguson, 1991; Trentham *et al.,* 2008). These cracks tend to increase in length and diameter as the fruit expands during the season and

will eventually form an interconnected network, together with an increase in the permeability of lenticels, as the season progresses (Trentham *et al.,* 2008). Due to a larger absorptive surface area of bigger fruits, late season foliar applications provide the fruit with more applied Ca compared to earlier applications (Schlegel and Schönherr, 2002). However, late season Ca foliar applications only penetrate the fruit surface by a few millimeters (Hanekom, 1975). The highly variable permeability between fruit lenticels and cracks also result a less uniform distribution of Ca in the outer cortex, which, to a certain extent, explain the varying distribution of bitter pit lesions in this region (Schlegel and Schönherr, 2002). Although early season foliar applications provide the fruit with less external total Ca, these sprays have been proven to be more efficient in reducing bitter pit incidence in specific instances (Neilsen and Neilsen, 2002; Schlegel and Schönherr, 2002; Neilsen *et al*., 2005; Lötze and Theron, 2007; Lötze *et al*., 2008).

1.3.3 Adjuvants

Surfactants are commonly used in conjunction with foliar Ca applications to increase its spreading properties and penetration by decreasing the surface tension of the spray solution (Harker and Ferguson, 1991). Some surfactants interact with the cuticle by increasing its permeability or by dilating the cuticle pores (Harker and Ferguson, 1991), but the efficiency of different surfactants vary greatly with regard to formulation and the respective cultivar involved (De Villiërs and Hanekom, 1977; Harker and Ferguson, 1991). Adding a surfactant can however, result in an increase in run-off, thereby reducing the Ca retained on the fruit surface as well as effective penetration (De Villiërs and Hanekom, 1977; Mason, 1979; Harker and Ferguson, 1991). Some surfactants have been proven to be successful in reducing the incidence in bitter pit

when applied in conjunction with foliar Ca (Reid and Padfield, 1975). The effective increase in the penetration of Ca of foliar products can also be enhanced by changing its point deliquescence when applied in combination with specific adjuvants (Blanco *et al*., 2010).

1.4 Quantifying Ca content of the fruit

1.4.1 Mineral analyses (whole fruit)

Bulk mineral analyses are currently used to determine the Ca status of the fruit in commercial practice. This type of analysis supplies information regarding the soluble Ca concentration of the whole fruit and is not suitable for determining the distribution of the Ca or the degree to which it is associated with specific fruit tissues, without increasing the number of analyses to comprise individual fruit sections as done by Wilkinson and Perring (1964), Terblanche, (1976) and Terblanche et al. (1979b). In these cases, samples were obtained at regular distances from the peel to the core, and did not reflect the influence of xylem Ca contributions.

1.4.2 Particle induced X-ray emission (PIXE)

PIXE has been used by Meyer *et al*. (1979, 1982) to determine both the distribution of Ca in apple fruit after storage, and illustrate how Ca is associated with pitted and non-pitted tissue. One of the main advantages of this method of elemental analyses, are a very high detection sensitivity of up to a few ppm (Meyer *et al*., 1982). Ca concentration was also reported

to rapidly increase towards the core of the fruit, possibly resulting from a higher prevalence of vascular bundles in this area (Meyer *et al*. 1982).

In order to quantify the contribution of different methods of Ca application to increase the Ca status of the fruit, it will be necessary to determine the exact locality of Ca deposition in the fruit during fruit development that is associated with the application strategies. This will be possible with PIXE technology.

1.5 Conclusion

The focus of the literature review was to update documentation on i) how different methods of Ca application and timing of application contribute towards the distribution of Ca in the fruit as well as ii) summarize exiting methodology to quantify Ca content/concentration in these locations.

Fruit physiological disorders associated with Ca have been reported to result from an inadequate distribution of fruit Ca, rather than a total deficiency (Bangerth, 1979; Saure, 2005). Therefore, we need to target Ca applications towards specific sections of the fruit during specific phenological stages of fruit development and via the suitable pathway.

Ca applied to the soil will enter the fruit through vascular bundles. This point of entrance is very different from Ca penetration through the fruit surface via foliar applications. Soil applications need to be synchronized with root growth phases and xylem activity in the fruit peduncle.

The efficacy of foliar applications is dependent amongst other on fruit surface that changes during fruit development. The timing of applications must therefore be determined by the aim of the application.

Although it is not applied at the time of utilization, Ca reserves play an important role in spring and also reach the fruit via the xylem vessels. Sufficient Ca reserves should be accumulated after harvest with the appropriate application method, to ensure the availability of sufficient Ca for bud and fruit development at the beginning of the season.

Ultimately, fruit quality will be the proof of sufficient Ca reaching susceptible tissues during the critical developmental phases, supplied via the different pathways. This needs to be quantified, at harvest and after storage, by visual inspection of fruit for e.g. bitter pit, evaluation of fruit quality parameters like fruit firmness, as well as nutrient composition (Ca) of the fruit. The results should indicate the efficacy of the different pathways to increase Ca concentration in the areas of insufficient Ca which can lead to cell disruption and death.

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Chapter 2: Paper 1

Evaluating the effectiveness of different calcium application strategies on the accumulation of calcium in apple fruit

Abstract

Orchards displaying calcium (Ca) deficiency, in spite of sufficient Ca in the soil and plant, is a common phenomenon worldwide. When localized Ca deficiencies occur, it affects the appearance of the fruit, with serious economic implications. A trial was conducted from 2007/8 to 2009/10 to re-evaluate the contribution of soil and foliar Ca applications to the final Ca concentration of 'Braeburn' fruit. $Ca(NO_3)_2$ was applied as six, weekly, foliar sprays (Calflo^{TM}) between approximately 21 and 70 days after full bloom (dafb), a soil application (Tropicote^{TM}) at fruit set or after harvest, and combinations thereof. Fruit Ca concentrations at 80 dafb were the highest in the treatments where foliar Ca was applied. In 2009/10, the average fruit Ca concentration, both at 80 dafb and at harvest, was the lowest for soil applications of Ca at fruit set. At harvest, fruit set soil applications resulted in significantly lower Ca concentrations in the treatments where i) a combination of soil applications at fruit set and after harvest were made and ii) only foliar sprays were applied. In this trial, mineral analysis for soluble Ca of the whole fruit (without the pips and stalk) was used to quantify the contribution of soil applied Ca. Results indicated that soil applications applied after harvest in the previous season and applications shortly after set in the current season did not increase Ca of the current season's fruit significantly, providing soil Ca levels were within the minimum requirements for apple trees. A possible explanation for this is that the tree regulates its Ca uptake via the roots when soil Ca is available in sufficient quantities. Root activity is essential for Ca uptake during the two root flushes and will impact on the efficiency of soil applied Ca. Fruit Ca concentrations were kept at

satisfactory levels at harvest (4.5 mg Ca.100g FW^{-1}) by applying a series of six foliar sprays early in the season.

Keywords: foliar sprays; soil application, mineral fruit content; *Malus domestica*

INTRODUCTION

Calcium translocation differs from translocation of other elements, as only small amounts are transported to the fruit compared to the large fraction incorporated into the foliage of the tree (Saure, 2002; 2005). A common appearance in fruit production is orchards, with adequate amounts of Ca in the soil, that still display localized Ca deficiencies in the fruit (Saure, 2005). Apples with low Ca concentrations are prone to developing physiological disorders like bitter pit, which influences the general appearance of the fruit (Rousseau, 1972; Ferguson and Watkins, 1983). When Ca related deficiencies like bitter pit and low fruit firmness occur, it is usually accompanied by substantial economic losses (Siddiqui and Bangerth, 1995; Saure, 2005). Some export markets only tolerate a bitter pit incidence of one percent in a consignment, which translates into one fruit in a count 100 carton (H. Griessel, Tru-Cape, P.O. Box 3772, Somerset West; personal communication).

Bitter pit symptoms are characterized as corky lesions that develop in the outer regions of the fruit flesh, typically become more pronounced through long term storage (Rousseau, 1972) and is often associated with 'Braeburn' apples. Extensive studies have been performed to explain the pathways of Ca in the tree and fruit, and to increase Ca uptake (Ferguson 1980; Ferguson and Watkins, 1983; Harker and Ferguson, 1991), but deficiencies still occur regularly.

At the beginning of the season, before the appearance of any leaves, there is a significant increase in the Ca concentration of the xylem sap, transporting Ca from the reserve tissues towards the shoots (Ferguson and Turner, 1981). According to Bradfield (1976) and Tromp (1979), this concentration of Ca in the xylem sap reaches a sharp peak about one week after bud break. This early remobilization of the Ca reserves of the tree can contribute up to 25% of the total Ca found in the new growth (Terblanche *et al*., 1979; Himelrick and McDuffie, 1983). At this time, the tree is supplied with Ca before any nutrients can be absorbed from the soil and root activity is still low (Saure, 2005). The net Ca import into the fruit, obtained from both the tree reserves and the soil solution, is the most efficient in early spring (Northern hemisphere) and Ca concentrations in the fruit peaks just after blossoming (Himelrick and McDuffie, 1983; Saure, 2005).

Most of the Ca that is absorbed by the roots moves towards the cell walls and intercellular spaces - the conducting tissue of the plant. This pathway is restricted in mature parts of the root by the presence of suberized Casparian bands, which develop on the outer surfaces of the endodermis layer surrounding the vasculature of the tree (White, 2001). Absorption of Ca from the soil solution therefore mainly occurs at newly developed root tips, were the endodermis is undeveloped (Himelrick and McDuffie, 1983). Active root growth is thus of the utmost importance for efficient uptake of Ca from the soil solution (Bangerth, 1979).

Two periods of active root growth typically occur during the growing season in apples: early spring and after harvest, during fall. The timing of root growth can vary considerably between

localities and thus, also the efficacy of timing of soil applications of fertilizers (Eissenstat *et al*., 2006). After absorption by the roots, Ca is mainly transported in the xylem of the plant (Himelrick and McDuffie, 1983). Applying Ca as a soil drench can increase fruit Ca concentration by increasing the Ca concentration in the xylem (Himelrick and McDuffie, 1983; Saure, 2005). This differs from foliar applied Ca, where the point of entry to the fruit cortex, is the peel (Harker and Ferguson, 1991).

As the season progresses, Ca concentration in the fruit is diluted due to a reduced Ca uptake and the rapid expansion of the fruit. Fruit Ca concentration will typically be highest in the skin and core, with the lowest values occurring in the outer cortex (Ferguson and Watkins, 1983; Himelrick and McDuffie, 1983). This is partly due to the natural occurring, more abundant distribution of xylem vessels towards the inner and upper parts of the fruit, as well as a relative higher growth rate of the outer cortex (flesh) (Rousseau, 1972; Hanekom, 1975; Ferguson and Watkins, 1989; Saure, 2005). As fruit expand, the inelastic xylem vessels rupture and eventually disintegrate, with implications for Ca supply to the fruit (Drazeta *et al*., 2004). Xylem disintegration occurs at 67 days after full bloom (dafb) for 'Granny Smith', but in 'Braeburn' apples, this happens earlier in the growing season (about 45 dafb), hence its predisposition to bitter pit incidence (Drazeta *et al*., 2004; Neilsen *et al*., 2005).

Ca sprays have been applied successfully to reduce Ca deficiencies and has been proven effective in reducing bitter pit, internationally and under South African conditions (De Villiers and Hanekom, 1977; Terblanche *et al*., 1980; Ferguson and Watkins, 1989; Saure 2002; Saure, 2005; Lötze and Theron, 2007). Six foliar sprays early in the season have effectively controlled

bitter pit in 'Golden Delicious' in South Africa (Lötze *et al*., 2008), and earlier work by Terblanche *et al*. (1980) reported a reduction of up to 16% bitter pit by applying late season foliar applications on 'Golden Delicious'. Additional Ca applied as foliar sprays are not remobilized from the leaves to other tree organs in any significant amounts (≤ 5%), and therefore only the Ca that is applied directly onto the fruit surface, will contribute towards the fruit Ca status (Hanekom, 1975; Saure 2002; Schlegel and Schönherr, 2002; Saure, 2005). Thus, the tissues just beneath the fruit peel (outer cortex), will benefit most from foliar applications (Hanekom, 1975). The rate of Ca penetration into the fruit is the highest early in the season (before 45 dafb), when the main pathway of penetration is via the trichomes and stomata on the fruit surface (Schlegel and Schönherr, 2002; Lötze and Theron, 2006). During the latter part of the season, most of the Ca enters the fruit through open lenticels and cracks, with uptake increasing as the season progresses when the fruit expand and cracks increase (Harker and Ferguson, 1991; Trentham *et al*., 2008). The highly variable permeability between lenticels later in the season often results in a less uniform distribution of Ca in the outer cortex, which explains the varying incidence of bitter pit lesions in the cortex to some extend (Schlegel and Schönherr, 2002). Although early season Ca sprays provide fruit with less total Ca when compared to later sprays, based on the smaller absorption capability of the fruit surface, these early applications have been proven more effective in reducing bitter pit incidence in some cases (Neilsen and Neilsen, 2002; Schlegel and Schönherr, 2002; Neilsen *et al*., 2005; Lötze and Theron, 2007; Lötze *et al*., 2008).

Ca foliar applications have additional advantages other than reducing bitter pit, e.g. maintaining fruit firmness during storage (Poovaiah, *et al.,* 1988; Siddiqui and Bangerth, 1995; Zocchi and

Mignani, 1995). Ca plays a structural role in the fruit cell wall and delays softening of fruits by inhibiting degradation of cell wall polymers (Diehl and Hamman, 1979; Sams and Conway 1984; Zocchi and Mignani, 1995; Fallahi *et al*., 2005), as well as reduces the occurrence of superficial pathogen infections. Thus, even fruit without prominent Ca deficiencies can benefit from additional Ca applications.

The aim of this study was to compare the efficiency of different combinations of soil- and/or foliar applied Ca regarding increasing fruit Ca concentration and improving fruit quality, focusing on the different mechanisms of Ca translocation that supply Ca to the fruit during different phenological stages of fruit growth. Once the most efficient application method and timing is determined, these results regarding application of Ca as $Ca(NO_3)_2$ for 'Braeburn' apples, will be communicated to the commercial industry.

MATERIALS AND METHODS

The field trial was conducted on a commercial farm, Eriskay, in the Vyeboom area (34˚ 3' 9.10" S; 19˚ 8' 20.30") over three consecutive growing seasons (2007/8 to 2009/10). Mature, full bearing 'Braeburn' apple trees, on rootstock M793 planted at a tree spacing of 4 x 1.5 m were used for the trial. $Ca(NO_3)_2$ was applied both as foliar spray (Calflo^{TM}) and soil application (Tropicote^{TM}) at 675 ml $100L^{-1}$ and 300 kg ha^{-1} respectively, per application, to represent five treatments as listed in Table I.

Six foliar applications (Calflo^{TM}) were applied weekly between approximately 21 and 70 dafb. Applications were made with a motorized knap sack sprayer until run-off. Per application,

approximately 1.7 L water was applied per tree, with no wetting agents. Applications were made during the early mornings (before 11h00) to enhance Ca uptake, which is known to decrease at temperatures above 21˚C. Soluble Tropicote™ pellets were used for soil applications after fruit set and/or after harvest (Table I). These applications changed to a split application on later dates after root studies started in season 2 (Table I). Standard applications of nitrogen, phosphorus and potassium (Turbo31™) were broadcasted onto the soil surface for all treatments in accordance to the commercial fertilizer program. All fertilizer products were supplied by YARA-Western Cape. The trial was conducted as a randomized complete block design with six replicates per treatment that each consisted of three tree plots. Each experimental unit was separated by buffer trees within the row, and adjacent rows by buffer rows, to reduce the effect of spray drift. Root growth was also monitored throughout the season. The site was visually evaluated for the presence of active root tips on a weekly basis from March 2009 to March 2010. Five composite soil samples (one per treatment) were collected at a depth of 30 cm before the trial commenced (August 2007) and again in September 2009.

At harvest (March) and about 80 dafb (December), leaf samples (30 leaves from the middle of a one-year-old shoot) and fruit samples (six fruit of similar size) were collected per block from both sides of the tree. Fruit were specifically selected from spurs on two-year-old wood to reduce variability. The whole fruit, without the pips and core, was analyzed. Dormant buds and flowers (1 g fresh weight (FW)) were also sampled before bud break and at fruit set respectively, for 2008/9 and 2009/10 (Table I). In addition, two-year-old shoots were sampled (separated into wood, bark and xylem sap samples) one week after bud break and analysed for Ca during 2008/9 and 2009/10. All bud-, bark-, wood-, xylem sap, flower-, fruit- and leaf mineral analyses were

carried out by a commercial laboratory. CAL labs (Pty) Ltd, (Somerset West) was used during the first season (2007/8) until 80 dafb 2008, and Bemlab (Pty) Ltd, (Strand) thereafter from harvest 2008/9 to harvest 2009/10.

Harvest sampling of fruit was aimed at pre-optimum maturity (≤20% starch breakdown) to enhance the incidence of bitter pit. Fruits of approximately similar size were selected randomly for both maturity indexing and evaluation after storage. Maturity evaluation was done on five (2007/8) and ten (2008/9 and 2009/10) fruit per block. Destructive sampling for this purpose was done at the Department of Horticultural Science, Stellenbosch University. An electronic fruit size measurer (EFM) and an electronic a scale was used to determine fruit size and mass respectively. Firmness was measured via a fruit texture analyzer (GÜSS Manufacturing (Pty) Ltd, (Strand). Fruit colour was determined according to colour charts both in terms of over (Deciduous Fruit Board (Pty) Ltd: set A44, Colour chart for 'Braeburn') and ground colour (Unifruco Research Services (Pty) Ltd: Colour chart for apples and pears). The percentage of starch breakdown was also assessed according to a chart (Unifruco Research Services (Pty) Ltd: Starch conversion chart (circular types), pome fruit). Fruits were cut diagonally and painted with a one percent iodine solution before visual inspection. A composite fruit sample was juiced for measurements of total soluble solids (TSS) and titratable acidity (TA). Acidity was determined by titration of the juice against a 0.1 $mol.L^{-1}$ sodium hydroxide solution in a Metrohm 760 sample changer. Total soluble solids (TSS) were determined with digital refractometer (ATAGO CO.LTD, ATAGO model PR 32). Twenty fruit per block were stored for two months at -0.5°C and then assessed for fruit quality after one day at approximately 22°C using the same parameters as with maturity indexing.

A separate evaluation to determine the incidence of bitter pit was also conducted after two months' cold storage at 0°C on approximately a hundred fruits per block, also harvested at the same time. Fruit were inspected individually for superficial bitter pit lesions and classified as either with or without bitter pit.

Analyses of the data were performed using the Statistical Analyses System (SAS) software (SAS Institute Inc, 2004, Cary, NC). Analyses of variance were performed using the general linear model procedure (GLM) to determine significant differences between treatments. Variances between treatments were established as significant at ten percent. A logit transformation was used to calculate the significance differences for bitter pit and lenticel break down incidence (Snedecor and Cochran, 1997).

RESULTS AND DISCUSSION

Dormant buds

Dormant buds were sampled at the beginning of September 2008 and 2009, before bud break and before Ca applications for the new season started. Ca that is transported to the bud at this early stage of the season should originate only from the remobilization of Ca reserves, as root growth has not yet started (Ferguson 1980; Saure, 2005; Eissenstat *et al*., 2006). Ca concentration in the xylem should now start to increase towards the peak reached at one week after bud break (Bradfield, 1976; Tromp, 1979). However, no significant differences in Ca concentration were found in dormant reproductive buds either in 2008/9 or 2009/10 (Table II). Average Ca concentrations for all treatments were slightly higher in 2008/9 (3.2% Ca) than in 2009/10 (3.0%

Ca), but this can possibly be ascribed to a change in laboratory from CAL labs Pty Ltd, (Somerset West) to Bemlab (Pty) Ltd, (Strand). At this stage (season 2) all treatments were applied twice, thus results reflect a sound comparison between the treatments. Nevertheless, no clear differences in bud Ca concentration were found between the different treatments and therefore presumably the Ca reserve status of the buds.

Flowers after set

The first flower analysis occurred in October 2007. This was before any treatments were applied. Mineral analyses for Ca showed no significant differences between treatments (Table III). Ca concentration was reported to peak just after blossom (fruit set) (Himelrick and McDuffie, 1983). The average Ca concentrations in the flowers just after set in 2009/10 (0.96% Ca) was almost double that of 2007/8 (0.46% Ca) and 2008/9 (0.45% Ca), but this is probably partly due to a change in laboratory from CAL labs Pty Ltd, (Somerset West) to Bemlab Pty Ltd, (Strand). This data also did not reflect differences in reserve Ca concentrations of the treatments, due to soil applications.

Fruitlets at 80 dafb

During the first season (2007/8), at 80 dafb, all six foliar sprays were applied, as well as one fruit set soil application. The fruitlet analyses at 80 dafb did not show any significant differences for Ca concentration between these treatments in 2007/8 or 2008/9, in spite of an additional soil application after harvest and fruit set, as well as another series of foliar sprays by 80 dafb in season 2 (2008/9). However, for fruitlets at 80 dafb in season 3 (2009/10), the treatment where only a soil application at fruit set was applied, showed a significantly lower Ca concentration

than the treatments consisting of (i) only foliar sprays and (ii) both soil applications at fruit set and after harvest (Table IV). In 2007/8 and 2008/9, higher fruitlet Ca concentrations (not significantly different from soil applications at fruit set) were found in two of the treatments where foliar sprays were applied, compared to the average. These concentrations were negatively correlated with fruit mass at 0.66 and 0.76 respectively (Figure 1). This confirms earlier findings that Ca concentration decreases as fruit size increases, due to a dilution affect (Saure, 2005). This may also indicate the lack of response to soil applied Ca early in the season, due to a lack of root activity at the time of application during the first two seasons. It also shows that Ca foliar applications, just before the analysis was performed, were accurately quantified by mineral analysis approximately two weeks (80 dafb) after the last application. In 2009/10, the Ca concentration at 80 dafb did not correlated with fruit size. This may be an indication that all treatments were finally applied at the optimal time for Ca absorption (2009/10), and that the treatments now reflect increases in the Ca status of the fruit based on additional Ca applications, irrespective of fruit size. The treatment where only foliar sprays were applied resulted in the highest Ca concentration in season 3 (2009/10) and this was significantly higher than the treatments with soil Ca application after fruit set, after harvest and soil application after harvest and foliar applications. The treatment where foliar sprays were combined with a post-harvest application of Ca did not differ significantly from the individual soil application treatments (Table IV). However, both treatments where foliar sprays were applied showed a trend towards the highest fruit Ca content (irrespective of the absolute concentration, Table IV) for all three seasons (Figure 2). The Ca concentrations of the fruits of 2008/9 were much higher than those sampled in 2007/8 and 2009/10. In 2008/9, spring was relatively cold and wet and this resulted in protracted flowering with varying full bloom dates and fruit sizes. Unintentionally, bigger

fruitlets (> 50 g.fruit^{-1}) were sampled at the calculated 80 dafb for 2008/9, containing higher Ca concentrations than expected at this stage (Table IV). However, if the fruit sizes for 2008/09 are adjusted to the average size at 80 dafb of the previous two seasons (average 30 g.fruit^{-1}), the fruitlet Ca concentrations are similar and show the same trends than those of the previous seasons (Figure 3).

Results for fruitlet Ca concentrations at 80 dafb indicate that foliar applications early during the season increase the Ca concentration in 'Braeburn' apples, confirming results on other cultivars (Neilsen and Neilsen, 2002; Schlegel and Schönherr, 2002; Neilsen *et al*., 2005; Lötze and Theron, 2007; Lötze *et al*., 2008). Soil applications around 40 dafb (present season) and after harvest (previous season) does not seem to be as effective in increasing fruit Ca at this early stage of fruit growth, compared to foliar applications just before the analyses. These results imply that mainly the Ca that is applied directly onto the fruit surface via a foliar application contributes to the fruit Ca status quantified at 80 dafb, because the contribution from the soil via the xylem could not be quantified here, and the fraction that is absorbed by the leaves after foliar application becomes relatively fixed and does not contribute additional Ca to the fruit (Hanekom, 1975; Saure, 2005). The noted inadequacy of soil applications during the first two seasons to increase the Ca content of the fruitlets determined at 80 dafb could have been a result of these treatments not being performed at the correct time of root growth flushes, as visually determined in 2009/10. This agrees with literature stating that Ca not supplied within the critical periods of active root growth, will not be absorbed from the soil (White, 2001; Saure, 2005; Eissenstat *et al*., 2006).

Another possible explanation regarding the lack of response to soil applied (after set) Ca to fruitlets at up to 40 dafb, lies in the functionality of the xylem in the fruitlet. If most of the xylem vessels are ruptured in 'Braeburn' apples at 45 dafb as implicated by Drazeta *et al*. (2004), soil applied Ca during the current season will not reach the fruit of the current season in significant amounts, if root growth and Ca applications only occurs after 40 dafb. Developing fruits will thus mainly depend on Ca from the reserves stored in tissues from the previous season. Therefore, the purpose of the soil applications after set (first root flush) and after harvest (second root flush), should be aimed at increasing the reserve Ca status of the tree, more so than the present season's fruit in the case of cultivars like 'Braeburn' that shows early disintegration of the xylem. However, the contribution of Ca via soil applications could not be quantified satisfactorily with the methodology used in this study for fruitlets 80 dafb.

Fruits at harvest

Mineral analyses of fruit Ca concentration at harvest only differed significantly between treatments at the end of the third season (2009/10). Fruits with the lowest Ca concentration (5.9 mg.100g FW^{-1}) resulted from the treatment where only a soil application after fruit set was applied. This was significantly lower than the treatments where i) only foliar sprays (7.3 mg.100g FW^{-1}) and ii) a combination of fruit set and after harvest soil Ca was applied (7.4 mg.100g FW^{-1}) (Table V). The same trend was observed for Ca concentration of fruitlets at 80 dafb in the same growing season (Figure 4). Thus, results again confirm the major contribution of foliar applications towards the fruit Ca concentration, compared to Ca supplied via soil applications during the previous autumn and present spring.

As expected, the Ca concentration was higher in fruits sampled at 80 dafb (average 9 mg.100g FW^{-1}) than those sampled at harvest (average 7 mg.100g FW^{-1}). This is in agreement with previous findings where Ca concentration decreased throughout the season as fruit expands (Himelrick and McDuffie, 1983; Saure 2005).

The effect of soil Ca applications only became visible after three seasons of treatment (2009/10), as shown by a change in trends in Ca concentration in season 3 compared to the previous ones. This was the case for fruits sampled at both 80 dafb and at harvest. The sudden change in Ca concentrations can be ascribed to either: (i) soil Ca not being applied at the critical periods of root growth for the first two growing seasons as quantified during season 3, (ii) a delayed effect from soil Ca applications over two seasons, since 'Braeburn' fruit is initiated approximately 14 months before harvest or (iii), the possible contribution of the reserve Ca of the tree that could not be quantified in this study. In this trial, during 2009/10 we noticed that root flushes occurred at different periods than proposed by information on 'Granny Smith' (Dept Horticultural Science, University of Stellenbosch) as shown in Figure 5 (Graph drawn up form information contained in 'Grondslagen van de fruitteelt'). White root tips were first observed late November in 2009 (around 40 - 80 dafb) at this specific site ('Braeburn' on a heavy, clay soil) – compared to October (around full bloom) according to the 'Granny Smith' information. These roots are essential in significant Ca absorption from the soil (White, 2001; Eissenstat *et al*., 2006). During the first two seasons, when monitoring for the presence of white roots did not take place, soil Ca was applied at fruit set in October and after harvest in May. It is therefore possible that Ca uptake during these seasons was not optimally synchronized with active root growth, which can explain the lack of effect of these treatments at this time. Although mineral analyses were done

by two different labs between 2007/8 and 2009/10, levels of fruit Ca were comparable in 2007/8 and 2008/9. This was in spite of the much higher Ca concentration applied to the tree via a soil application (35.5 g) compared to the total foliar application of six sprays to the foliage and fruit (7.78 g) (Table VI).

Fruits sampled at harvest in 2009/10 (6.84 mg.100g FW^{-1}) had an overall higher average Ca concentration than those from the previous two seasons (4.54 and 5.11 mg.100g FW^{-1}), and this is not due to a change in laboratory. A consistent increase in fruit Ca occurred during consecutive seasons in most treatments. Except for the soil application after harvest and at fruit set in 2007/8, fruits from all other treatments and seasons showed satisfactory levels of fruit Ca at harvest, being above the critical threshold of 4 – 4.5 mg.100g FW^{-1} for good fruit quality as described by Neilsen *et al.* (2005) an Terblanche (1985) respectively. Bitter pit is also highly unlikely to occur when fruit Ca levels are above 4.5 mg.100g FW^{-1} (Terblanche, 1985). This level was achieved with all treatments for seasons 2 and 3. These levels of fruit Ca were also achieved by applying only six early foliar applications early during the season as opposed to a minimum of 10 sprays that are presently applied commercially to reduce the incidence of bitter pit. A possible contributor to the increased Ca concentrations of the fruit at harvest, is the decrease in fruit size as the yield increased during the trial period (Figure 6), confirming the relationship between fruit size and Ca concentration (Saure, 2005). The steady increase in Ca concentrations could also be due to a build up effect of soil applied Ca (reserves) in the case of these treatments. Alternatively a carry-over effect from foliar applications during consecutive seasons could have resulted, where a percentage of Ca may have been relocated from the leaves at the end of the season. According to Rousseau (1972), the fraction of Ca that is relocated from

the leaves to the tree structure is only about five percent. Thus, this small percentage may supply the fruits and/or reserve tissues with Ca, when considering the contribution of the leaf area of approximately 20-25 leaves per fruit. However, this is in contrast to earlier findings (Hanekom, 1975; Saure 2005) that foliar applied Ca will not contribute significant amounts to the reserves of the tree.

Leaf analyses

As expected, when sampled at 80 dafb (approximately 14 days after the last foliar application), all the treatments where foliar sprays were applied had higher (although not significant) leaf Ca concentrations compared to the other treatments that received no foliar applications for all three seasons (Table VII). Leaf analyses results at harvest showed no significant differences in the Ca concentration between the treatments in 2007/8 and 2008/9. In 2009/10, Ca concentrations were the highest where foliar sprays were applied and these treatments also were significantly higher than where soil applications were made individually (Table VIII). Leaf analyses results in 2009/10 showed the same trend at 80 dafb as at harvest, where leaf Ca concentrations were higher following foliar application compared to soil applications. At this stage of sampling, three fruit set and two soil applications after harvest had been made, along with three series of foliar sprays. Results indicate a higher efficiency of foliar applications in supplying the leaves with Ca compared to soil applications. As mentioned before, soil applied Ca was probably not optimally absorbed by the roots due to incorrect timing of the applications during seasons 1 and 2. When taken into account that the post harvest and fruit set soil applications during season 3 were applied at the correct times of active root flush, one would expect a total increase in leaf Ca concentration at the end of at least season 3, with additional Ca being supplied via the xylem,

from the roots either as reserve Ca after harvest (Apr/May 2009), or Ca during the growing season, applied during early summer (Nov/Dec 2009), as the xylem in the leaf petioles was intact at least until the leaf analysis was done at harvest. At this stage, translocation of Ca to the reserve tissues from leaves from all treatments is taken as similar – thus, this could not explain the lack of differences between treatments either. Thus, in spite of a functional xylem connection of the roots to the leaves, additional soil applied Ca (high concentration Ca) when root activity was present during the early summer flush and the period after harvest, a low concentration of foliar applied Ca treatments still resulted in a significantly higher Ca concentration in the leaves at harvest.

Xylem sap-, bark- and wood analyses

There were no significant differences between treatments in terms of Ca concentrations of the wood, bark and xylem sap in 2008/9 and 2009/10 (Table IX, X). In 2009/10, xylem sap showed the same trend for the Ca concentration as for fruit Ca concentrations at harvest and 80 dafb for the different treatments (Figure 7). This may indicate the importance of reserves in the tree at this early stage and the influence thereof on the Ca concentration of the fruit at 80 dafb and at harvest, via translocation from Ca in the xylem, from reserves in the wood.

Fruit quality

No significant differences in fruit firmness occurred in 2007/8 and 2008/9 (Tables XI, XII, XIII and (XIV). In 2009/10, there were significant differences in fruit firmness between treatments, both at harvest and after storage (Table XV and XVI). However, firmness was inversely correlated with fruit size at R^2 values of 0.76 (harvest) and 0.60 (storage), and could thus not be

directly attributed to Ca concentration. When fruit size was used as a covariate, there were no significant differences in firmness any more (Table XVII). As expected with the ripening of the fruit, firmness decreased through storage.

No significant differences occurred in terms of ground colour for fruits analyzed both at harvest and after storage for any of the seasons (Tables XI - XVI). This indicated that there was no direct effect of the $Ca(NO_3)_2$ formulation on green colour degradation. In terms of red colour, significant differences were found between treatments after storage in 2007/8, but this could not be attributed to the different treatments that were applied (Tables XII). Red colour of 'Braeburn' is a quality parameter, and the average values ranging between 2.1 and 4.5, show that colour development was not sufficient for commercial export in this trial, due to pre-mature harvesting of the samples. As reflected in the percentage of starch breakdown at harvest, fruits were harvested more mature in 2009/10 (36.8 - 47.9%) than in 2008/9 (10.3 - 18.6%) and 2007/8 (14 - 30.3%), however fruits were still sampled during the pre-optimum stage (Tables XI, XIII, XV). No significant differences for bitter pit incidence occurred between treatments for any of the seasons (logit BP), but the incidence was relatively low (max 2.99%) (Tables XII, XIV, XVI), although, still too high for some export markets (1 % threshold level).

Significant differences occurred between treatments in terms of TSS at harvest (2008/9 and 2009/10) and after storage in 2008/9, as well as for TA after storage (2008/9), but this cannot be attributed to a direct effect of the treatments (Tables XIII, XIV, XV).

CONCLUSION

Our results showed that early season foliar applications of $Ca(NO_3)_2$ contributed more soluble Ca to the fruit and leaves of 'Braeburn' trees at 80 dafb than soil applications. This is the critical period when bitter pit is initiated (Schlegel and Schönherr, 2002) and thus when additional Ca can reduce bitter pit incidence. In the present trial, the average Ca concentration from fruits in all treatments was above 4.5 mg.100g FW^{-1} at harvest, the threshold for low bitter pit incidence (Terblanche, 1985) and high fruit quality, in 2008/9 and 2009/10. This shows that satisfactorily Ca levels at harvest can be maintained by applying six foliar sprays early in the season.

In 2009/10, after three seasons of Ca applications, Ca concentrations were the lowest following the soil applications after fruit set. These results may indicate that soil applications of Ca after fruit set contribute less Ca to fruit of the current season than expected, and should be regarded as an application for reserve Ca for the following season's crop instead in the case of 'Braeburn' apples. The amount of Ca transported from the reserve tissues in the xylem sap one week after bud break has been shown to have a significant influence in the Ca concentration of the fruit. The post harvest soil Ca application in our study did not contribute more Ca towards the reserve status of the tree, (as quantified by Ca concentration of two-year-old shoots (dormant buds and flowers at set), at the beginning of the following seasons), compared to foliar sprays. It seems possible that the tree has an internal mechanism of regulating the Ca supply of the reserve status and translocation thereof to the fruit, by not absorbing excess Ca (above a critical level) when applied to the soil, as indicated by no differentiation between treatments in xylem Ca concentration.

Based on the preceding evidence of the study on 'Braeburn' apples and $Ca(NO_3)_2$ applications, our recommendation to improve fruit Ca concentration of the current season's fruit, at harvest, are the following:

i) Maintain soil Ca status: Soil Ca applications after 40 dafb (first root flush) and after harvest (second root flush) can be used to achieve this, providing active root growth occurs. This can be used together with a mineral soil analysis regarding the soluble Ca concentration, indicating whether the critical Ca levels defined by industry standards for apple trees are met. Monitoring of active root growth is also highly recommended before soil applications are carried out, as these critical periods typically vary between cultivars, seasons and localities. These applications will be focused on building Ca reserves for the following season's fruit.

ii) Apply early foliar sprays to supply fruit with additional Ca in the critical period of fruit development, with regard to bitter pit and fruit quality, where necessary. These applications will be applicable to increase the Ca status of the present crop.

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TABLE I

Fertilization, sampling and evaluation dates indicating yield for the trial from 2007/8 to 2009/10

Treatments applied			
Soil application after harvest			
Soil applications after harvest and after fruit set			
Soil application after harvest and foliar sprays after fruit set			
Foliar sprays after fruit set			
Soil application after fruit set			
Application dates of Calcium Nitrate	2007	2008	2009
After harvest	-	23.05.2008	Split 14.05.2009/22.05.2009
Fruit set	26.10.2007	10.11.2008	Split 4.11.2009/13.11.2009
Foliar spray 1	6.11.2007	6.11.2008	13.11.2009
Foliar spray 2	13.11.2007	14.11.2008	20.11.2009
Foliar spray 3	19.11.2007	21.11.2008	26.11.2009
Foliar spray 4	3.12.2007	27.11.2008	2.12.2009
Foliar spray 5	7.12.2007	4.12.2008	10.12.2009
Foliar spray 6	10.12.2007	10.12.2008	17.12.2009
Sampling and evaluation dates	Date 1	Date 2	Date 3
Soil sampling	[1]26.10.2007	-	[2]28.10.2009
Bud, bark, wood and xylem sampling	-	[1]01.09.2008	[2]14.09.2009
Full bloom	12.10.2007	20.10.2008	16.10.2009
Flower sampling	[1]25.10.2007	[1]29.10.2008	[2]20.10.2009
Leaf/Fruit sampling (80dafb)	[1]20.12.2007	[1]22.12.2008	[2]07.01.2010
Leaf/Fruit sampling (harvest)	[1]18.03.2008	[2]26.03.2009	[2]23.03.2010
Maturity indexing	19.03.2008	27.03.2009	25.03.2010
Evaluation after storage	27.05.2008	03.06.2009	02.06.2010
Season	2007	2008	2009
Commercial yield t.ha-1	67	96	97

[1] CAL PTY LABORATORIES
[2] BEMLAB PTY LTD

TABLE II

Calcium concentrations of dormant reproductive 'Braeburn' buds sampled September 2008 and 2009

Treatment	2008	2009
	%	
Soil application after harvest	3.220 ns	2.922 ns
Soil applications after harvest and after fruit set	3.373	3.063
Soil application after harvest and foliar sprays after fruit set	3.073	3.062
Foliar sprays after fruit set	3.223	2.843
Soil application after fruit set	3.197	3.095
Sign level	0.2559	0.2236
LSD (5%)	0.26	0.26

TABLE III

Calcium concentrations of 'Braeburn' apple flowers sampled October 2007, 2008 and 2009

Treatment	2007	2008	2009
		%	
Soil application after harvest	0.473 ns	0.470 ns	0.958 ns
Soil applications after harvest and after fruit set	0.450	0.477	0.983
Soil application after harvest and foliar sprays after fruit set	0.467	0.443	0.943
Foliar sprays after fruit set	0.458	0.417	0.895
Soil application after fruit set	0.448	0.433	1.027
Sign level	0.6443	0.4694	0.5370
LSD (5%)	0.04	0.08	0.16

TABLE IV

Calcium concentrations of 'Braeburn' apple fruitlets sampled at 80 dafb in December/January 2008, 2009 and 2010

Treatment	2008	2009	2010
		mg.100g FW^{-1}	
Soil application after harvest	8.160 ns	13.578 ns	9.117 bc
Soil applications after harvest and after fruit set	7.225	10.858	10.117 ab
Soil application after harvest and foliar sprays after fruit set	8.702	16.183	9.067 bc
Foliar sprays after fruit set	8.108	15.545	10.683 a
Soil application after fruit set	6.600	12.210	8.383 c
Sign level	0.5931	0.7528	0.0063
LSD (5%)	2.92	9.53	1.22

TABLE V

Calcium concentrations of 'Braeburn' apple fruits sampled at harvest in March 2008, 2009 and 2010

Treatment	2008	2009	2010
		mg.100g FW^{-1}	
Soil application after harvest	6.218 ns	5.967ns	6.717 ab
Soil applications after harvest and after fruit set	3.490	4.633	7.433 a
Soil application after harvest and foliar sprays after fruit set	4.262	4.667	6.783 ab
Foliar sprays after fruit set	4.390	4.817	7.333 a
Soil application after fruit set	4.347	5.450	5.933 b
Sign level	0.2106	0.3729	0.0781
LSD (5%)	2.34	1.62	1.13

TABLE VI

Total amount of Ca added throughout one whole season per tree for each treatment

Treatment	Active Ca	Concentration	Product applied	Total Ca / season
Soil application after harvest	18.8 % (Tropicote™)	NA	189 g/tree	35.5 g /tree
Soil applications after harvest and at fruit set	18.8 % (Tropicote™)	NA	378 g/tree	71.0 g /tree
Soil application after harvest and foliar sprays after fruit set	12% (Calflo™) and 18.8% (Tropicote™)	6.75ml/L @ 1.6L/tree (6 sprays)	64.8 ml + 189 g	43/28 g / tree
Foliar sprays after fruit set	12% (Calflo™)	6.75ml/L @ 1.6L/tree (6 sprays)	64.8 ml	7.78 g /tree
Soil application at fruit set	18.8% (Tropicote™)	NA	189 g	35.5 g /tree

TABLE VII

Calcium concentrations of 'Braeburn' apple leaves sampled at 80 dafb in December 2008, 2009 and January 2010

Treatment	2008	2009	2010
		%	
Soil application after harvest	1.133 ab	1.023 b	1.270 c
Soil applications after harvest and after fruit set	1.088 b	0.993 b	1.377 bc
Soil application after harvest and foliar sprays after fruit set	1.165 ab	1.080 ab	1.517 ab
Foliar sprays after fruit set	1.278 a	1.250 a	1.575 a
Soil application after fruit set	1.093 b	0.978 b	1.388 abc
Sign level	0.0824	0.0940	0.0307
LSD (5%)	0.15	0.22	0.20

TABLE VIII

Calcium concentrations of 'Braeburn' apple leaves sampled at harvest in March 2008, 2009 and 2010

Treatment	2008	2009	2010
		%	
Soil application after harvest	1.660 ns	1.588 ns	1.843 bc
Soil applications after harvest and after fruit set	1.608	1.645	1.975 ab
Soil application after harvest and foliar sprays after fruit set	1.668	1.603	2.013 a
Foliar sprays after fruit set	1.683	1.687	1.993 ab
Soil application after fruit set	1.613	1.702	1.790 c
Sign level	0.8071	0.7402	0.0201
LSD (5%)	0.16	0.21	0.15

TABLE IX

Calcium concentrations of the xylem sap, wood and bark of two year old 'Braeburn' shoots, 1 week before bud break in 2009

Treatment	Xylem sap	Bark	Wood
	mg/L	%	%
Soil application after harvest	16.03 ns	2.60 ns	0.94 ns
Soil applications after harvest and at fruit set	23.36	2.66	0.91
Foliar sprays after fruit set	47.27	2.82	1.00
Sign level	0.2133	0.5517	0.7164
LSD (5%)	38.26	0.45	0.24

TABLE X

Calcium concentrations of the xylem sap, wood and bark of two year old 'Braeburn' shoots, 1 week after bud break in 2010

Treatment	Xylem sap mg/L	Bark %	Wood %
Soil application after harvest	134.590 ns	2.535 ns	0.990 ns
Soil applications after harvest and at fruit set	201.150	2.890	1.033
Soil application after harvest and foliar sprays after fruit set	156.200	2.945	1.096
Foliar sprays after fruit set	185.060	2.713	0.813
Soil application at fruit set	118.720	2.740	0.918
Sign level	0.5778	0.3277	0.2446
LSD (5%)	97.16	0.40	0.28

TABLE XI

Maturity indexing results of 'Braeburn' apple fruits sampled and analyzed at harvest in March 2008

Treatment	STARCH %	FIRM kg	DIAM mm	MASS g	[1]BACKCOL	ACID % Malic	TSS %
Soil application after harvest	14.0 b	9.34 ns	67.9 ns	145.5	2.32 ns	0.65 ns	10.32 ns
Soil applications after harvest and after fruit set	21.0 ab	9.22	68.1	148.1	2.35	0.61	10.47
Soil application after harvest and foliar sprays after fruit set	21.3 ab	9.09	69.9	156.3	2.45	0.66	10.51
Foliar sprays after fruit set	24.7 ab	9.08	68.9	149.3	2.63	0.59	10.26
Soil application after fruit set	30.3 a	8.94	69.9	156.7	2.25	0.61	10.33
Sign level	0.1078	0.4800	0.0506	0.1066	0.1678	0.1741	0.8640
LSD (5%)	11.88	0.47	1.64	10.09	0.33	0.06	0.55

[1]BACKCOL – fruit ground colour according to colour chart

TABLE XII

Quality evaluation results of 'Braeburn' apple fruits sampled at harvest 2008 and stored at 0.5˚C for approximately three months

Treatment	STARCH	FIRM	DIAM	MASS	[1]BACKCOL	[2]REDCOL	ACID	TSS	[3]BP	[4]BP
	%	kg	mm	g			% Malic	%	logit	%
Soil application after harvest	79.3 ns	8.04 ns	69.3 ns	156.3 ns	2.40 ns	2.53 b	0.62 ab	12.32 ns	-3.84 ns	2.03
Soil applications after harvest and after fruit set	86.8	7.85	70.0	153.7	2.50	2.48 b	0.58 b	11.88	-4.82	0.52
Soil application after harvest and foliar sprays after fruit set	82.8	7.87	71.4	159.5	2.53	2.33 b	0.65 a	11.93	-4.37	1.06
Foliar sprays after fruit set	83.6	8.04	69.6	150.4	2.49	3.31 a	0.61 ab	11.90	-4.03	1.32
Soil application after fruit set	86.6	7.84	71.4	160.5	2.67	2.59 b	0.60 ab	11.88	-4.10	2.39
Sign level	0.3353	0.7108	0.2558	0.8514	0.2373	0.0707	0.1884	0.2474	0.2668	
LSD (5%)	8.23	0.41	2.48	21.25	0.23	0.71	0.05	0.46	0.95	

[1]BACKCOL – fruit ground colour according to colour chart
[2]REDCOL – fruit over colour according to colour chart
[3]BPLogit = LOG((Bitter pit fruit + 0.5)/(Total no. fruit – Bitter pit fruit + 0.5))
[4]BP% - number of bitter pit fruit in the sample as percentage

TABLE XIII

Maturity indexing results of 'Braeburn' apple fruits sampled and analyzed at harvest in March 2009

Treatment	STARCH %	FIRM kg	DIAM mm	MASS g	[1]BACKCOL	[2]REDCOL	ACID % Malic	TSS %
Soil application after harvest	15.3 ns	9.43 ns	68.9 b	141.4 c	2.20 ns	2.13 ns	0.62 ns	11.68 a
Soil applications after harvest and after fruit set	11.9	9.40	69.2 ab	142.8 bc	2.18	2.83	0.62	11.28 ab
Soil application after harvest and foliar sprays after fruit set	15.3	8.96	71.4 a	155.5 a	2.20	2.27	0.63	11.12 ab
Foliar sprays after fruit set	18.6	9.04	70.8 ab	153.5 ab	2.25	2.95	0.60	10.93 b
Soil application after fruit set	10.3	9.16	68.8 b	139.6 c	2.18	3.25	0.60	10.85 b
Sign level	0.6631	0.2251	0.0889	0.0224	0.8676	0.1140	0.8834	0.0529
LSD (5%)	12.22	0.50	2.26	11.41	0.16	0.95	0.07	0.58

[1]BACKCOL – fruit ground colour according to colour chart
[2]REDCOL – fruit over colour according to colour chart

TABLE XIV

Quality evaluation results of 'Braeburn' apple fruits sampled at harvest 2009 and stored at 0.5˚C for approximately three months

Treatment	STARCH %	FIRM kg	DIAM mm	MASS g	[1]BACKCOL	[2]REDCOL	ACID % Malic	TSS %	[3]BP logit	[4]BP %
Soil application after harvest	72.05 ns	8.38 ns	65.6 c	149.5 ns	2.41 ns	2.68 ns	0.62 ns	12.97 a	-4.52 ns	1.52
Soil applications after harvest and after fruit set	66.7	8.56	66.3 c	155.1	2.61	2.86	0.62	12.82 ab	-4.70	0.67
Soil application after harvest and foliar sprays after fruit set	80.0	8.26	68.7 a	155.8	2.49	2.49	0.62	12.55 bac	-4.01	2.99
Foliar sprays after fruit set	79.3	8.40	68.2 ab	235.5	2.73	3.24	0.63	12.08 c	-4.29	1.20
Soil application after fruit set	79.5	8.35	65.36 c	214.4	2.47	3.27	0.61	12.33 bc	-4.31	1.20
Sign level	0.2160	0.5264	0.0079	0.2100	0.7141	0.3343	0.9545	0.0301	0.8063	
LSD (5%)	13.05	0.36	2.10	92.84	0.51	0.91	0.05	0.58	1.21	

[1]BACKCOL – fruit ground colour according to colour chart
[2]REDCOL – fruit over colour according to colour chart
[3]BPLogit = LOG((Bitter pit fruit + 0.5)/(Total no. fruit – Bitter pit fruit + 0.5))
[4]BP% - number of bitter pit fruit in the sample as percentage

TABLE XV

Maturity indexing results of 'Braeburn' apple fruits sampled and analyzed at harvest in March 2010

Treatment	STARCH %	FIRM kg	DIAM mm	MASS g	[1]BACKCOL	[2]REDCOL	ACID % Malic	TSS %
Soil application after harvest	36.8 ns	9.27 a	66.3 c	128.6 c	2.9 ns	2.55 ns	0.61 ns	13.73 a
Soil applications after harvest and after fruit set	38.2	9.25 a	66.1 c	135.0 bc	2.90	3.22	0.60	13.22 ab
Soil application after harvest and foliar sprays after fruit set	47.9	8.78 b	69.6 a	152.5 a	2.76	2.62	0.63	13.10 ab
Foliar sprays after fruit set	40.4	9.06 ab	67.4 bc	142.3 ab	2.74	3.07	0.62	13.39 a
Soil application after fruit set	43.3	8.71 b	68.0 ab	144.5 ab	2.66	2.90	0.62	12.65 b
Sign level	0.4000	0.0396	0.0097	0.0018	0.1224	0.7213	0.7614	0.0661
LSD (5%)	12.70	0.44	12.80	1.65	0.21	1.17	0.05	0.73

[1]BACKCOL – fruit ground colour according to colour chart
[2]REDCOL – fruit over colour according to colour chart

TABLE XVI

Quality evaluation results of 'Braeburn' apple fruits sampled at harvest 2010 and stored at 0.5˚C for approximately three months

Treatment	FIRM	DIAM	MASS	[1]BACKCOL	[2]REDCOL	ACID	TSS	[3]BP	[4]LB	[5]BP	[6]LB
	kg	mm	g			% Malic	%	logit	logit	%	%
Soil application after harvest	7.45 a	65.85 ns	134.85 ns	3.00 ns	4.50 ns	0.60 ns	13.75 ns	-5.40 ns	-5.07 ns	0.00	0.48
Soil applications after harvest and after fruit set	7.27 ab	65.9	134.0	2.99	4.13	0.57	13.55	-4.95	-4.83	0.95	0.75
Soil application after harvest and foliar sprays after fruit set	7.00 b	67.5	143.2	3.10	3.29	0.60	13.27	-5.16	-4.72	0.17	1.1
Foliar sprays after fruit set	7.21 ab	67.0	140.8	3.06	4.37	0.60	13.40	-5.35	-4.71	0.00	0.5
Soil application after fruit set	7.29 ab	66.6	140.3	3.10	4.04	0.59	13.28	-5.34	-4.45	0.00	1.32
Sign level	0.0549	0.5198	0.4517	0.8913	0.1537	0.7812	0.6023	0.6384	0.8565		
LSD (5%)	0.28	12.92	2.15	0.31	1.01	0.05	0.72	0.69	1.15		

[1]BACKCOL – fruit ground colour according to colour chart
[2]REDCOL – fruit over colour according to colour chart
[3]BP Logit = LOG((Bitter pit fruit + 0.5)/(Total no. fruit – Bitter pit fruit + 0.5))
[3]BP LB = LOG((Lenticel break down pit fruit + 0.5)/(Total no. fruit – Lenticel break down fruit + 0.5))
[5]BP % - number of bitter pit fruit in the sample as percentage
[6]LB % - number of lenticel break down fruit in the sample as percentage

TABLE XVII

Firmness values of 'Braeburn' apple fruits sampled at harvest in March 2010 and analyzed at harvest and after three months of storage at 0.5˚C using fruit mass as a covariate

Treatment	Harvest kg	After storage kg
Soil application after harvest	9.26 ns	7.42 ns
Soil applications after harvest and after fruit set	9.24	7.23
Soil application after harvest and foliar sprays after fruit set	8.77	7.03
Foliar sprays after fruit set	9.07	7.23
Soil application after fruit set	8.72	7.30
Sign level	0.0581	0.1216
LSD (5%)	0.45	0.28

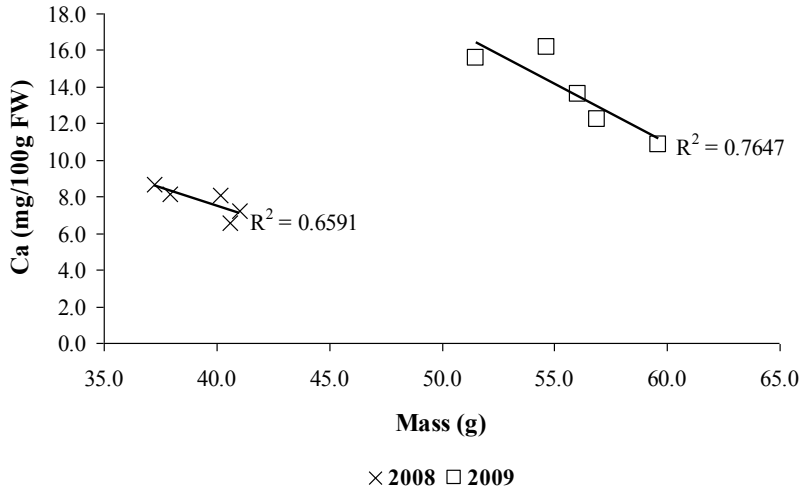

FIG. 1

Ca concentration of 'Braeburn' apple fruitlets, sampled at 80 days after full bloom, in relation to fruitlet mass for 2008 and 2009.

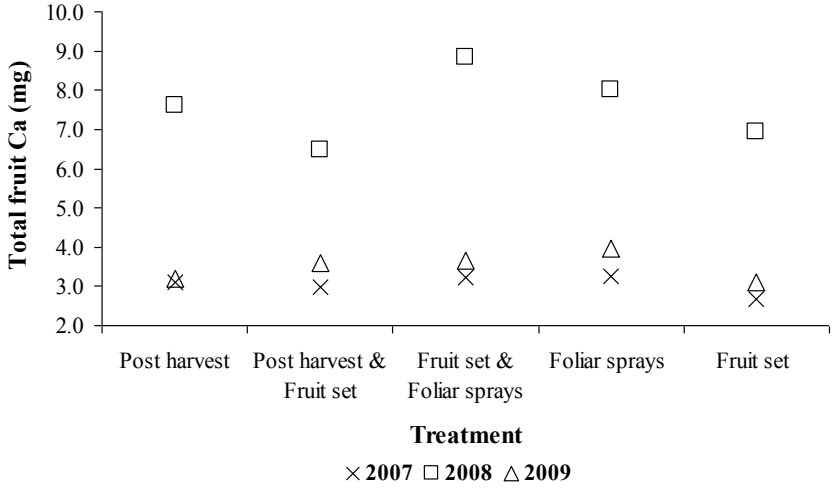

FIG. 2

Total soluble Calcium content of 'Braeburn' apple fruitlets, sampled at 80 days after full bloom, according to the treatments applied for three seasons.

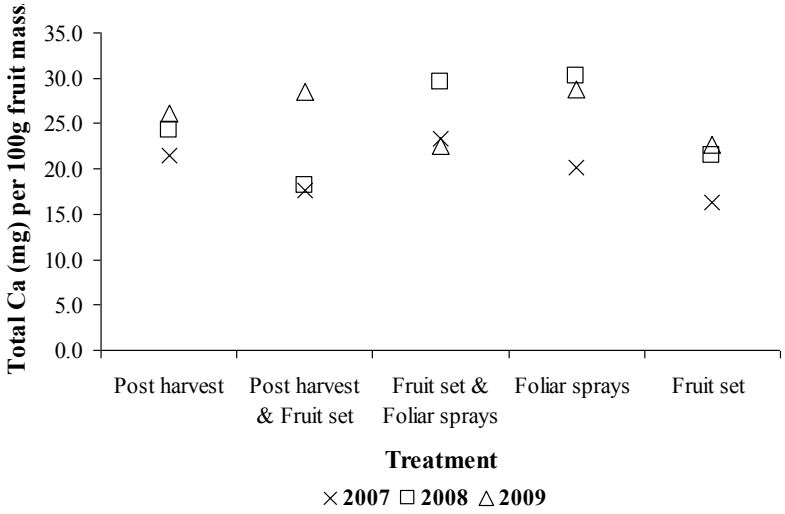

FIG. 3

Total Ca content per 100g fruit mass of 'Braeburn' apples at 80dafb, according to treatments applied (March 2008, 2009, 2010)

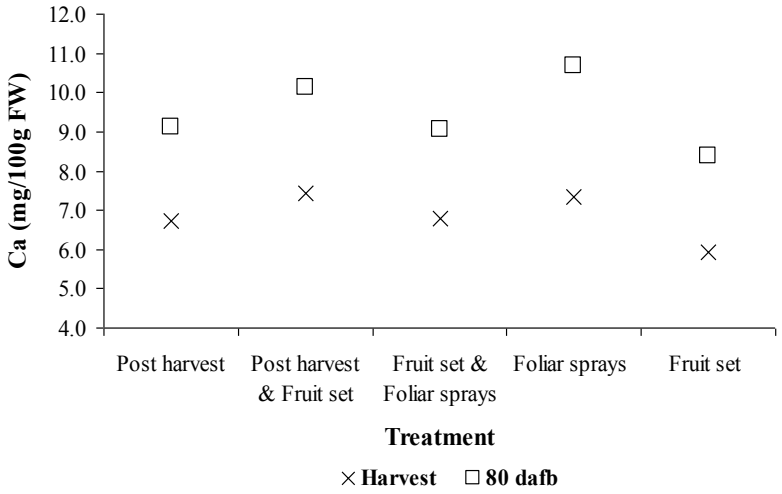

FIG. 4

Ca concentration of 'Braeburn' apple fruits, sampled at 80 days after full bloom and at harvest, for all treatments, after three seasons of application 2010.

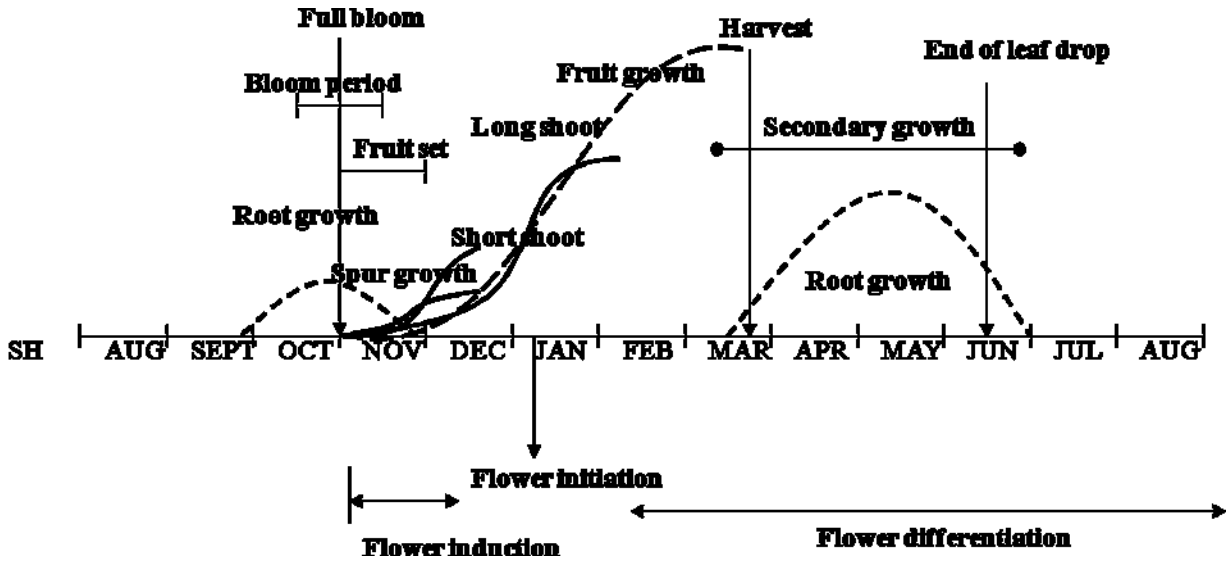

FIG. 5

Phenological development of 'Granny Smith' apple. (Dept of Horticultural Science, Stellenbosch University, based on 'Grondslagen van de fruitteelt').

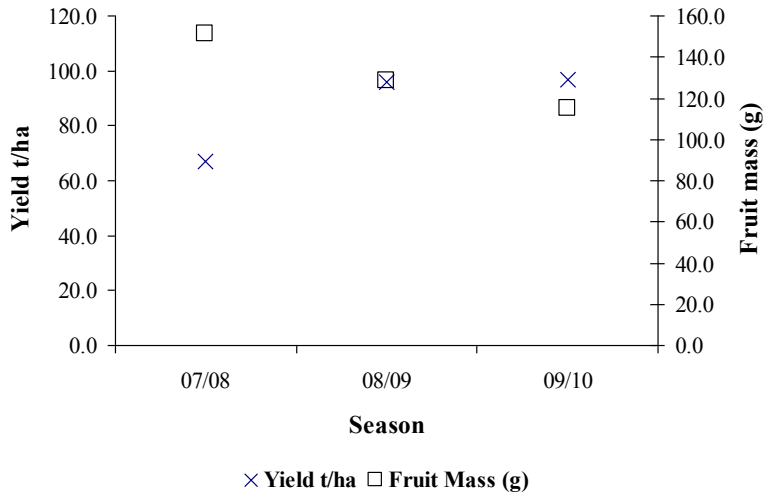

FIG. 6

Total yield and average fruit mass of 'Braeburn' apples at harvest, per treatment, for three seasons (March 2008, 2009, 2010).

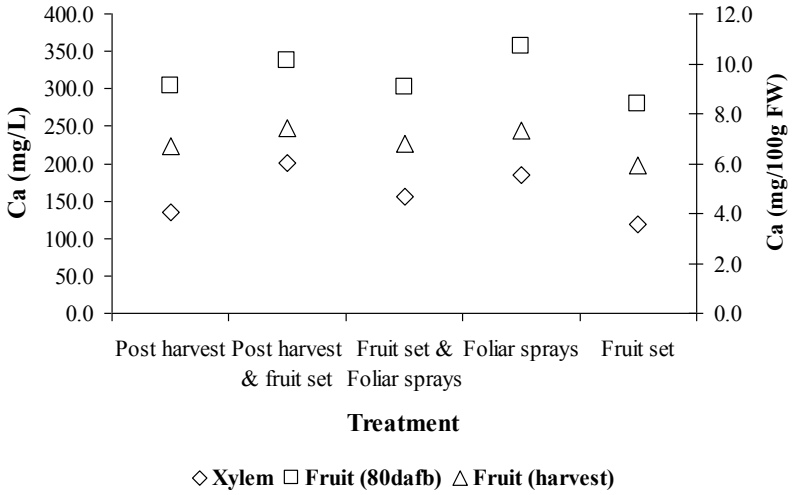

FIG 7.

Ca concentration of the xylem sap and 'Braeburn' apples sampled at one week after bud break and 80 dafb / harvest respectively.

Chapter 3: Paper 2

Determining the efficiency of new foliar calcium formulations on apple fruit calcium concentration and quality

ABSTRACT

Calcium (Ca) sprays are applied internationally as a standard practice to enhance fruit quality and control fruit physiological disorders such as bitter pit and low fruit firmness. In the first trial one, the effectiveness of two new formulations of foliar Ca products (Foliar GS™ and Foliar GG™) was evaluated in relation to existing formulations of Calflo™ and Calcimax™ regarding an increase in fruit Ca and reduction of bitter pit. All treatments were applied as eight, weekly sprays from 90 dafb and only differed in terms of concentration and amount of active Ca in the formulation. Calflo™ resulted in fruits with significantly higher Ca concentrations when compared to the Foliar GS™ and Foliar GG™ treatments. The concentration and formulation of the products played a significant role in this trial. In the second trial, three different treatments (Calflo™, Messenger™ and at commercial treatment consisting of Calcinit™, Maxiboost™, Spraybor™, Zincmax™ and Foliwett™) were applied in different concentrations and at different times throughout the season, according to product specification. The commercial treatment consisted of the highest Ca concentration and was applied later in the season compared to the other treatments. It resulted in fruit with the highest Ca concentration, but fruit firmness was affected negatively. Application of Messenger™ did not increase fruit Ca concentration, but increased fruit colour. Late season sprays prove more efficient in increasing fruit Ca concentration when compared to early season sprays, confirming earlier results. The bitter pit percentage in both trials were very low (>3%) and could not be related to any Ca treatment.

Keywords: foliar products; mineral fruit content; *Malus domestica*

1. INTRODUCTION

Most orchards have adequate amounts of Calcium (Ca) in the soil, yet localized Ca deficiencies within the fruits of these trees still develop and this is often accompanied with large economic implications when problems with low fruit firmness or physiological disorders like bitter pit occur (Siddiqui and Bangerth, 1995; Saure, 2005). These problems develop mainly because of a poor distribution of Ca within the tree and fruit, with most of the absorbed Ca being transported primarily towards the leaves that represent the major sink (Bangerth, 1979).

The Ca that is absorbed from the soil, is translocated towards the upper parts of the tree, almost exclusively via the xylem (Himelrick and McDuffie, 1983). These vascular tissues comprise conducting vessels that extend from the roots up to the leaf margins or fruits (Saure, 2005). As the foliage develops, it competes increasingly with the fruits as sinks for Ca, because the leaves are much higher transpiring organs than the fruits. Thus, more water will be transported through the xylem towards the leaves (Bangerth, 1979). The decreasing rate of Ca import into the fruit cannot satisfy the need of the expanding fruit and Ca concentrations in the flesh will drop as the season progresses (Saure, 2005). This explains partly why localized Ca deficiencies can occur, even in Ca rich soils, and also why Ca fertilization has often been ineffective in reducing these problems (Bangerth, 1979). According to an anatomical study, most primary xylem vessels disintegrate early in the season (45 dafb) for 'Braeburn' apples (Drazeta *et al*., 2004) and this leads to a reduced uptake of Ca into the fruit (Neilsen *et al*., 2005).

Fruits with a Ca concentration below 4 - 4.5 mg.100g FW^{-1}, run the risk of developing bitter pit (Terblanche, 1985; Ferguson and Watkins 1989). Symptoms are characteristically corky, brown lesions that develop in the outer regions of the flesh (Ferguson and Watkins, 1989; Hanekom, 1975). This is largely due to the disruption of groups of cortex cells just beneath the skin of the fruit (Ferguson and Watkins, 1989; Rousseau, 1972). A relatively higher growth rate (cell expansion), accompanied with a restricted presence of vascular bundles further contribute to a dilution of Ca in the outer cortical flesh (Ferguson and Watkins, 1989; Saure, 2005).

Ca is also known to affect other fruit quality factors like increasing fruit firmness and extending storage life (Bangerth, 1979; Poovaiah *et al*., 1988; Zocchi and Mignani, 1995). This is because Ca plays a structural role in the cell wall and delays cell wall degradation that results to softening of the fruit (Diehl and Hamman, 1979; Sams and Conway 1984; Zocchi and Mignani, 1995; Fallahi *et al*., 1997). The effect of foliar Ca applications on fruit firmness is not always visible at harvest, but through storage it allows the fruit to keep its desirable texture for a longer period (Siddiqui and Bangerth, 1995).

Ca sprays have commonly been used in orchards, both internationally and in South Africa, to reduce these deficiencies and plays an important role in controlling bitter pit (Rousseau, 1972; De Villiers and Hanekom, 1977; Terblanche *et al*., 1980; Ferguson and Watkins, 1989; Neilsen and Neilsen, 2002; Saure, 2005; Lötze and Theron, 2007; Lötze *et al*., 2008). Foliar Ca needs to be applied directly onto the fruit surface as only five percent of the total Ca has been observed to be relocated out of the leaves (Rousseau, 1972; Saure, 2005). The regions in the outer cortex of

the fruit benefit most from foliar sprays and it is here where symptoms of bitter pit are mostly expressed (Hanekom, 1975). During the latter part of the season, Ca mainly enters the fruit through open cracks and lenticels (Harker and Ferguson, 1991). These cracks, along with lenticel permeability, tend to increase as the season progresses (Trentham *et al*., 2008). More Ca will also be absorbed through a larger fruit surface later in the season compared to the early part of the season (Mason, 1979; Schlegel and Schönherr, 2002; Lötze and Theron, 2006).

Although late season sprays proved more effective in increasing the total Ca status of the fruit, these sprays have been shown to only penetrate the fruit by a few millimeters, and most of the Ca is then located within the free spaces and does not contribute to general functioning of the cells (Hanekom, 1975; Ferguson and Watkins, 1983; Saure, 2005; Trentham *et al*., 2008). The highly selective yet variable permeability between fruit lenticels later in the season can also result in a less uniform distribution of Ca in the outer fruit cortex and this can have some implications in controlling bitter pit (Schlegel and Schönherr, 2002). Foliar Ca penetrates through the peel at a much higher rate when applied before 45 dafb due to the presence of trichomes on the surface of a young and developing fruit (Schlegel and Schönherr, 2002; Lötze and Theron, 2006; Blanco *et al*., 2010).

The role of the formulation of the product in Ca absorption has been discussed by Schlegel and Schönherr (2002) in their work with $CaCl_2$ and $Ca(NO_3)_2$. The point of deliquescence influenced the penetration efficiency of the formulation and this was unique for each formulation. Based on this, the penetration of the Ca in different formulations decreased from $CaCl_2$ to $Ca(NO_3)_2$. Research by Joubert (2007) also confirmed that the difference between four Ca products (Calflo,

Calcimax, Ca acetate and Ca fulvate) with different formulations, active Ca concentrations and application frequencies resulted in the expected difference in total active Ca applied per tree during the season and thus, Ca concentration in the fruit at harvest and bitter pit incidence in 'Golden Delicious' apples. Thus, emphasizing the effect of Ca formulations on fruit quality.

Surfactants can sometimes be added to products to reduce the surface tension of the spray droplets and increase the spreading properties of the application over the fruit surface (Harker and Ferguson, 1991). It can therefore be used to increase the absorption of the application, but the efficiency of the surfactant largely depends on its concentration and formulation (De Villiërs and Hanekom, 1977; Harker and Ferguson, 1991). In some cases, surfactants decreased the uptake of Ca by increasing run off and this reduces the total weight of Ca that is retained on the fruit surface (De Villiërs and Hanekom, 1977; Mason 1979; Harker and Ferguson, 1991). A surfactant known as Lecthin™ has been used successfully in conjunction with $Ca(NO_3)_2$ as a post harvest dip to reduce bitter pit (Reid and Padfield, 1975). Lecthin™ was applied as a pre harvest spray to evaluate its effectiveness when used with $Ca(NO_3)_2$. Apart from applying surfactants, it has also been shown by Blanco *et al*. (2010) that the addition of adjuvants to a pre-harvest spray solution can improve the retention and penetration of Ca to the cuticle, by altering its point of deliquescence, and the distribution of Ca within the spray droplet. Significant results in reducing bitter pit have been achieved by the addition of adjuvants to a Ca spray solution (Blanco *et al*., 2010).

Messenger™ consists of a harpin protein that is known to activate defense mechanisms within the plant (Dong *et al*., 1999). By applying this protein as a foliar spray, positive effects in terms

of fruit firmness and colour have been reported in the UK, as well as additional positive effects on Ca transport (product brochure, personal communication, J. Holohan, Plan Health Care (UK), Ltd.).

The contribution of a foliar Ca application depends on various factors e.g. time of application and the chemical formulation of the product. The aim of this trial was to evaluate the efficiency of new formulations of foliar Ca products against existing products regarding an increase in the fruit Ca status, reduction on bitter pit incidence and effect on fruit quality during long term storage.

2. MATERIALS AND METHODS

2.1 Trial 1

The first trial was conducted on a commercial farm, Eriskay, in Vyeboom (34˚ 3' 9.10" S; 19˚ 8' 20.30" E). Full bearing, mature 'Braeburn' apple trees, planted on M793 and with a spacing of 4 m x 1.5 m were selected for this purpose. Five different formulations of foliar Ca products were applied to the trees during the growing season of 2008/2009 and compared to a control treatment of no applications (Table 1). Products were applied according to the timing and concentrations supplied by the manufacturer (UAP-Plaaskem).

The efficiency of new formulated Ca-products (Foliar GS^{TM} and Foliar GG^{TM}) in improving fruit quality and increasing fruit Ca was evaluated in relation to existing formulations of Calflo^{TM} and Calcimax^{TM}. Products were applied as eight weekly foliar sprays from 90 - 140 days after full bloom (dafb) with motorized knapsack sprayers (Table 2). The last spray was applied two weeks

before harvest. Water from the site was used and all sprays were applied at approximately 1.6 $L.tree^{-1}$. Sprays were applied during the early morning to enhance uptake. The trial was conducted as a randomized complete block design, with six replicates per treatment consisting of single tree blocks. The experimental units were separated by buffer trees to reduce the possibility of spray drift.

Six fruit and twenty leaves per block were sampled on both sides of the tree before and after the treatments were applied. Fruits were sampled exclusively from spurs on two year old wood to reduce variability. Mineral analyses of fruits and leaves were carried out by the Department of Soil Science, Stellenbosch University (before treatments) and Bemlab (Pty) Ltd, Strand (after treatments). Whole fruit were analyzed excluding the pips and core. External Ca residues contained on the surface were removed beforehand by washing the fruits with a 1% v/v HCl solution and then rinsing it with de-ionised water. Samples were analyzed via the standard method using the ICP-OES (Inductively Coupled Plasma – Optical Emission Spectrometer) procedure together with a nitrogen analyzer.

A composite sample of 25 fruit per treatment, of approximately the same size, was taken at harvest for maturity indexing that took place in March of 2009. Destructive sampling took place at the department of Horticultural Science, Stellenbosch University. An electronic scale and fruit size measurer (EFM) were used to determine fruit mass and size respectively, while fruit firmness was measured using a fruit texture analyzer (FTA) (GÜSS Manufacturing (Pty) Ltd, Strand). Fruits were halved diagonally and the surface of the calyx end stained with a 1% iodine solution to indicate the percentage starch breakdown that occurred. Charts were used to visually

determine fruit over- and background colour as well as the percentage of starch breakdown and russet. These were 1: (Deciduous fruit board (Pty) Ltd) Colour chart for Braeburn set A44, Russet chart for Golden Delicious and 2: (Unifruco research services (Pty) Ltd) Colour chart for apples and pears, Starch conversion chart (circular types), pome fruit.

Fruits were cut into wedges and juiced to obtain a composite sample. A digital refractometer (ATAGO CO.LTD, ATAGO model PR 32) was used to determine the total soluble solid (TSS) content. Additional juice was also titrated against a 0.1 $mol.L^{-1}$ sodium hydroxide solution, in a Metrohm 760 sample changer, to determine the titratable acidity.

2.2 Trial 2

The second trial was conducted over a single growing season on full bearing, mature 'Golden Delicious' apple trees on a commercial farm, Queen Anne, also in Vyeboom (34° 2' 41.10" S; 19° 12' 53.20" O) with a historical record of a 20 percent bitter pit incidence. Five different foliar products were applied from full bloom (2009/10/19) until harvest (2010/02/21) and compared to a control treatment (no application) as listed in Table 1. Beside the application of Ca containing products, a harpin protein (Messenger^{TM}) product was also included in the treatments to evaluate its effect on fruit quality and Ca concentration. Treatments were applied according to the timing and concentrations specified by the supplier of the product.

The sprays were applied with motorized knap sack sprayers in the early mornings to enhance uptake. The dates of application of each treatment are listed in Table 2. No additional adjuvants were added and water from the site was used for the applications. The trail was constructed as a

randomized complete block design with six treatments consisting of five blocks each and these were separated by buffer trees to reduce the possibility of spray drift. Each block consisted of two trees.

Six fruit per block was sampled at 80 dafb and at harvest from both sides of the tree. Fruits were specifically sampled from spurs of two year old wood to reduce variability. Ca mapping of fruit sectors were done at iThemba LABS at 80 dafb via particle induced x-ray emission. Maps were obtained by repetitive scanning of 3.0 MeV focused proton beam on areas ca. 2 mm x 2 mm. Fruits that were sampled at harvest were sent to Bemlab (Pty Ltd, Strand) for mineral analyses. Individual fruits were analyzed without the pips and core via the procedure described in trial 1. All sampling dates are indicated in Table 3.

Maturity evaluation was done on twenty fruit per block both at harvest and after a storage period of approximately two months at 0.5°C. Fruits of similar size were selected for this purpose. Maturity indexing and evaluation was carried out according to the methods described in trial 1. A separate analysis to determine the incidence of bitter pit was carried out on approximately a hundred fruits per replicate after two months of storage at -0.5° C. Fruits were classified as either having bitter pit or being totally free of any external symptoms.

2.3 Statistical analyses

Analyses of the data were performed using the Statistical Analyses System (SAS) software. Analyses of variance were performed with the general linear model procedure (GLM). Variances

between treatments were considered as significant at a probability of ten percent. A logit transformation was used on the bitter pit data (Snedecor and Cockran, 1997).

3. RESULTS

3.1 Trial 1

3.1.1 Mineral analyses

There were significant differences in terms of fruit K (potassium) and Mg (magnesium) before application of the treatments and these were used as a covariate in further analyses (Table 4). No significant differences in terms of fruit Ca were obtained before the treatments were applied.

Trees that received Calflo^{TM} had the highest Ca values in the fruit at harvest 6.08 mg.100g FW^{-1} (Table 5). This is also the only application that resulted in higher Ca values than the control treatment. Trees that received new formulations of Foliar GS^{TM} and Foliar GG^{TM} showed significantly lower fruit Ca concentrations than the Calflo^{TM} treatment at $P \leq 0.1$ (Table 5). Where Lecithin^{TM} was applied with Calcimax^{TM}, fruit Ca levels remained more or less the same as the treatment where Calcimax^{TM} was applied without a surfactant (Table 5). Ca levels decreased towards harvest from approximately 7 mg.100g FW^{-1} to approximately 5 mg.100g FW^{-1} and that is probably due to dilution of the expanding fruit. The unforeseen change in laboratory from US Department of Soil Science to Bemlab (Pty) Ltd, Strand could have also played a role in the difference of the actual Ca levels.

There were no significant differences in the Ca concentrations of the leaves both before and after the treatments were applied (Tables 6 and 7). Due to technical problems, no leaf phosphate analyses occurred before treatments were applied.

Fruit N (nitrogen) concentrations were the highest for applications of $Ca(NO_3)_2$ compared to the rest of the treatments. This was observed in three of the treatments: Calflo™, Calcimax™ and Calcimax™ with Lecithin™ (Table 5). Fruits treated with Calflo™ had significantly higher concentrations of N than the GS™, GG™ and control treatment (Table 5). It is also speculated that the formulation of Calcimax™ contains P, as fruits treated with this treatment had significantly higher concentrations of P than the control and GS™ treatments (formulation unknown).

3.2 Trial 2

3.2.1 Mineral analyses

Three additional commercial Ca applications were applied to the trial at 47, 54 and 61 dafb – which was not foreseen. Fruits that received commercial sprays had significantly higher concentrations of Ca, Zn (zinc) and B (boron) than the other treatments (Table 8). Messenger™ and Calflo™ resulted in slightly higher concentrations of fruit Ca than the control treatment, although these differences were not significant (Table 8). Trees that received applications of the commercial sprays or Calflo™ (containing NO_3^-) had higher concentrations of fruit N than the Messenger™ (contains harpin protein), but these amounts of N were lower than the control treatment and differences were non-significant.

3.2.2 Maturity evaluation

After three months' storage, fruits from the Messenger™ treatment had the highest firmness values of all treatments (Table 10). Although not significant, this was the only treatment where fruit firmness was improved compared to the control treatment. Messenger™ treated fruit also had the highest colour values (more yellow), both at harvest and after storage (1.58 and 3.96 respectively), and this was significantly higher than all the other treatments after storage (Table 10). The percentage starch break down was significantly higher for the commercial treatment at harvest (Table 9). After storage, fruits also had significantly lower firmness values for the commercial treatment. At harvest, there was no russet development for the commercial treatment (Table 9). After storage, the incidence of russet was significantly lower for fruits treated with commercial sprays compared to the other treatments. Both at harvest and after storage, the highest incidences of russet were observed for the control (1.8%, 4.28%) and Messenger™ (0.8%, 4.24%) treatments. Although non-significant, a decrease in russet incidence was observed for all treatments where relatively high amounts of $Ca(NO_3)_2$ was applied, compared to the control. TSS values after storage were significantly lower for fruits that received the commercial treatment (Table 10). This was unexpected, for this treatment also resulted in a significantly higher percentage starch break down at harvest (Table 9), indicating more mature fruit. Firmness decreased through storage as expected. Almost no bitter pit (<2%) was observed during 2009/10 compared to 20% in 2008/9 and no significant differences were observed for bitter pit incidence between treatments after storage (Table 10).

4. DISCUSSION

4.1Trial 1

4.1.1 Mineral analyses

Applications of Calflo™ performed the best in terms of increasing fruit Ca at harvest. Foliar GS™ and Foliar GG™ did not improve the Ca status of the fruit compared to existing formulations of Calcimax™ and Calflo™ ($Ca(NO_3)_2$). However, the total amounts of Ca added per tree by spraying Calflo™ (10.37g) is approximately double that of Calcimax™ (6.91g), GS™ (5.76g) and GG™ (5.76g) and this was probably the main contributor to the high Ca concentration in the fruit at harvest (Table 1). Calflo™ is also slightly more expensive compared to Calcimax™ (Table 11). Adding Lecithin™ to Calicmax™ did not significantly improve its uptake as fruit Ca concentrations were 5.00 mg.100g FW^{-1} and 5.05 mg.100g FW^{-1} for Calicmax™ and Calicmax™ + Lecithin™ respectively (Table 5). Calcimax™, GS™ and GG™ were applied at the same spray volume, concentration and timing, and number of applications (eight) (Table 1) and the amount of Ca added per tree is comparable (5.76 – 6.91 g $tree^{-1}$). However, results indicate that a product containing 12 % active Ca (Calcimax™) increased fruit Ca concentration more compared to products with only 10% active Ca (GS™ and GG™), when applied similarly, as expected. This is in agreement with existing literature (Lötze *et al*., 2008; Blanco *et al*., 2010), indicating that the efficiency of a Ca foliar product also depends on its formulation. Fruits from most treatments had satisfactory levels of Ca (> 4.5 mg.100g FW^{-1}) (Kotze 2001; Terblanche, 1985) required for good fruit quality, except for GS™ (4.15 mg.100g FW^{-1}). This partly explains the low bitter pit incidence we experienced (Terblanche, 1985; Ferguson and Watkins, 1989). Although treatments differed significantly in terms of fruit N and

Ca concentrations, no significant effect was observed in the fruit quality assessment (data not shown).

4.2 Trial 2

4.2.1 Mineral analyses

The commercial treatment showed an increase in fruit Ca, Cu, Zn and B and this due to the inclusion of $(CaNO_3)_2$, Zincmax™ and Spraybor™ in the treatment (Table 8). These products were successfully applied as a tank mix with a surfactant (Foliwett™). The total amount of Ca added per tree over the season was much higher in the commercial treatment (10.5 g.tree^{-1}) compared to the Calflo™ (7 g tree^{-1}), Messenger™ (1.9 g.tree^{-1}) and control treatments (1.9 g.tree^{-1}) (Table 1) and explains why fruits from the commercial treatment had the highest Ca concentrations at harvest. The Ca source in the commercial treatment (Calcinit™), is however much more expensive compared to Calflo™, both in terms of the total seasonal cost of application (labour excluded) and the cost per amount of Ca added (Table 11). Messenger™ did not result in any significant increase in fruit Ca at harvest. Although the Calflo™ treatment resulted in significant amounts of Ca being applied per tree, this did not increase fruit Ca as expected compared to the commercial treatment. The most important factor contributing to this difference in fruit Ca concentration between these treatments, is the timing of the sprays. Calflo™ was applied earlier in the season resulting in much lower Ca concentrations at harvest. These results are in agreement with various authors (Mason, 1979; Schlegel and Schönherr, 2002; Lötze and Theron, 2006) who found that late season foliar applications are more effective in increasing fruit Ca at harvest than early season applications.

4.2.2 Maturity evaluation

There are reports showing that Messenger™ increases sugar, firmness, colour and possibly would lead to a decrease in bitter pit incidence (product brochure). However, in this trial, TSS levels and firmness were not affected by applying Messenger™, as there was no significant difference in TSS between the Messenger™ and the control treatment, at harvest or post storage. Fruits had the highest colour values where Messenger™ was applied (harvest and post storage). This was significantly higher than all the other treatments after storage, indicating the effect on colour development, without enhancing maturity in the other parameters. Messenger™ did not affect bitter pit incidence and this is in agreement with Rosenberger *et al*. (2004). Fruits showed a decrease in firmness and increase in colour as ripening commenced during storage. Fruits from the commercial treatment were more mature, as starch breakdown was significantly higher at harvest and fruit firmness significantly lower after storage, when compared to the other treatments (Table 9 and 10). More mature fruit has been reported to be less susceptible to bitter pit incidence. Thus, the more mature fruit with a higher fruit Ca concentration of the commercial treatment contributed towards the low incidence of bitter pit at 0.88%. Russet incidence was reduced both at harvest and after storage for the treatments where relatively higher amounts of $Ca(NO_3)_2$ was applied (Commercial and Calflo™). It therefore seems as if foliar Ca sprays can decrease the incidence of russet when applied at the correct volume and concentration.

5. CONCLUSION

New formulations of Calcimax™ (Foliar GS™ and Foliar GG™) could not compete favourably with existing formulations of Calflo™ and Calcimax™ in increasing fruit Ca, when products were applied according to specifications from the suppliers (Trial 1). Calflo™ outperformed all

tested products in terms of fruit Ca concentration at harvest, in spite of having a similar active Ca percentage than Calcimax™. Calcimax™ resulted in fruits with higher concentrations of Ca at harvest when compared to GS™ and GG™ because of a higher amount of active Ca in its formulation. Adding Lecithin™ to Calcimax™ is not recommended, as the surfactant did not improve Ca uptake.

A commercial treatment (7 weekly applications of Calcinit™) performed the best and significantly increased fruit Ca concentrations compared to the other treatments when applied to 'Golden Delicious' trees. The commercial treatment did however showed negative effects on some fruit quality parameters, as it enhanced ripening and negatively affected fruit firmness at harvest. The application of Messenger™, at the recommended rate and timing, did not enhance natural occurring Ca transport to the fruit as quantified by a fruit mineral analysis at harvest, but did enhance colour development without affecting other fruit maturity parameters.

The timing and amount of Ca being applied to the tree seems to be the most important factors in determining fruit Ca concentration at harvest – with later applications resulting in a higher Ca concentration at harvest. The application of six weekly Calflo™ sprays during 2008/9, commencing from as early as 28 dafb, was found to maintain satisfactorily levels of fruit quality, did not show any signs of foliar or fruit damage and did not show bitter pit in 'Braeburn'apples. The amount of active Ca in the formulation of the product also plays a significant role, when the sprays are applied at the same concentration and timing, as it resulted in a higher final fruit Ca concentration at harvest. However, if a disorder is initiated early during the season, or Ca is required early in the season, the contribution of late applied Ca is questionable.

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TABLE 1

Concentration and specifications of the products for the treatments applied in trial 1 and 2 during 2008/2009 and 2009/2010 respectively.

Treatment	Concentration	Volume water	Sprays	Application Period	Active Ca	Ca/tree
Trial 1						
CalcimaxTM	31.5ml / 7L	1.6L/tree	8	90-140 dafb	12%	6.91g
CalcimaxTM and Li700TM	31.5ml and 8.75ml / 7L	1.6L/tree	8	90-140 dafb	12%	6.91g
CalfloTM	47.25ml / 7L	1.6L/tree	8	90-140 dafb	12%	10.37g
Foliar GGTM	31.5ml / 7L	1.6L/tree	8	90-140 dafb	10%	5.76g
Foliar GSTM	31.5ml / 7L	1.6L/tree	8	90-140 dafb	10%	5.76g
Control	no treatment					
Trial 2						
Treatments						
1.Control + (3 Ca sprays)						1.9g
2.MessengerTM + (3 Ca sprays)						1.9g
3.CalfloTM + (3 Ca sprays)						7g
4.Commercial + (3 Ca sprays)						10.5g
Control (no treatment)					0%	0g
MessengerTM	1.25g/5L	0.6L/tree	4	100% petal drop	0%	0g
				3 weeks after petal drop		
				2 weeks before commercial harvest		
				1 week before commercial harvest		
CalfloTM	62.7ml/10L	1L/tree	6	weekly (28 -63 dafb)	12%	4.5g
Commercial sprays		1.6L/tree	7	weekly (49 -91 dafb)	12%	8.6g
CalcinitTM	64g/10L					
MaxiboostTM	20ml/10L					
SprayborTM	5g/10L					
ZincmaxTM	8ml/10L					
FoliwetTM	0.5ml/10L					
(Ca-sprays)	64g/10L	1.6 L/tree	1	40 dafb	12%	1.2g
	64g/10L	0.45 L/tree	2	47 and 54 dafb	12%	0.7g

TABLE 2

Application dates of foliar products during 2008/2009 and 2009/2010.

Product	Application 1	Application 2	Application 3	Application 4	Application 5	Application 6	Application 7	Application 8
Trial 1								
All treatments	15.01.2009	23.01.2009	29.01.2009	05.02.2009	12.02.2009	20.02.2009	26.02.2009	05.02.2009
Trial 2								
MessengerTM	22.10.2009	10.11.2009	19.01.2010	02.02.2010				
CalfloTM Ca(NO3)2	17.11.2009	24.11.2009	01.12.2009	08.12.2009	15.12.2009	22.12.2009		
Commercial sprays	08.12.2009	15.12.2009	22.12.2009	29.02.2009	05.01.2010	12.01.2010	19.01.2010	
Ca sprays	17.11.2009	01.12.2009	15.12.2009					

TABLE 3

Full bloom- and sampling dates for 2008/2009 and 2009/2010.

	Trial 1	Trial 2
Full bloom date	16.10.2008	19.10.2009
Fruit sampling (80dafb)	08.01.2009	08.01.2010
Fruit sampling (harvest)	27.03.2009	22.02.2010
Maturity indexing (harvest)	27.03.2009	23.02.2010
Evaluation after storage	-	02.06.2010

TABLE 4

Mineral nutrient concentration for 'Braeburn' apples before treatment on ‘Braeburn’ (01/2009).

Treatment	N	P	K	Ca	Mg	MASS
	mg/100g fresh weight					g
CalcimaxTM + Li 700TM	196.38 ns	2.03 ns	115.58 a	7.61 ns	5.95 a	41.73 ns
CalfloTM	189.61	1.95	112.45 a	7.02	6.00 a	38.79
Foliar GSTM	172.44	1.78	95.87 b	7.10	4.99 b	39.00
Control	177.26	1.61	105.26 ab	7.45	5.51 ab	39.05
Foliar GGTM	178.34	1.93	105.45 ab	7.11	5.41 ab	41.11
CalcimaxTM	165.85	2.21	114.24 a	7.85	5.99 a	40.39
P>F	0.0883	0.3034	0.0379	0.6432	0.0351	0.3628
$LSD_{(5\%)}$	29.9230	0.5305	12.9360	1.1836	0.7050	3.3908

TABLE 5

Mineral nutrient concentrations after foliar applications for 'Braeburn' apples at harvest (03/2009).

Treatment	N	P	K	Ca	Mg	MASS
	mg/100g fresh weight					g
CalcimaxTM + Li700TM	54.00 ab	11.14 a	116.67 ns	5.05 ab	5.75 ns	133.68 ns
CalfloTM	59.50 a	9.56 ab	125.67	6.08 a	6.10	133.47
Foliar GSTM	46.00 b	7.67 b	109.33	4.15 b	5.30	131.20
Control	45.50 b	7.67 b	107.5	5.28 ab	5.67	122.77
Foliar GGTM	47.83 b	10.10 ab	112.83	4.67 b	5.63	131.17
CalcimaxTM	53.00 ab	11.03 a	120.83	5.00 ab	6.03	131.12
P>F	0.0484	0.0278	0.1568	0.0655	0.0817	0.6007
$LSD_{(5\%)}$	9.8582	2.5940	15.2620	1.2123	0.5680	13.5460

TABLE 6

Mineral nutrient concentrations for 'Braeburn' leaves before treatment (01/2009).

Treatment	N	K	Ca	Mg
			%	
CalcimaxTM + Li700TM	2.33 ns	1.270 ns	0.108 ns	0.25 ns
CalfloTM	2.45	1.14	0.10	0.23
Foliar GSTM	2.28	1.25	0.12	0.26
Control	2.35	1.23	0.11	0.26
Foliar GGTM	2.33	1.43	0.12	0.28
CalcimaxTM	2.40	1.22	0.11	0.27
P>F	0.8546	0.5877	0.3284	0.4585
$LSD_{(5\%)}$	0.2745	0.3236	0.0255	0.0577

TABLE 7

Mineral nutrient concentrations for 'Braeburn' leaves after foliar applications at harvest (03/2009).

Treatment	N	P	K	Ca	Mg
			%		
CalcimaxTM + Li700TM	2.560 ns	0.222 ns	0.832 ns	1.292 ns	0.294 ns
CalfloTM	2.548	0.124	0.730	1.242	0.258
Foliar GSTM	2.546	0.154	0.708	1.250	0.286
Control	2.542	0.125	0.648	1.090	0.290
Foliar GGTM	2.520	0.194	0.742	1.236	0.276
CalcimaxTM	2.463	0.168	0.723	1.218	0.288
P>F	0.7440	0.2823	0.2350	0.1188	0.4124
$LSD_{(5\%)}$	0.1466	0.10	0.1724	0.1588	0.049

TABLE 8

Mineral nutrient concentrations for 'Golden Delicious' apple fruit after foliar applications (at harvest 02/2009)

Treatment	N	P	K	Ca	Mg	Na	Mn	Fe	Cu	Zn	B	MASS
			mg/100g					mg/kg				g
control + 3(Ca sprays)	56.00 ns	11.81 ns	119.80 ns	5.50 b	6.40 ns	14.72 ns	1.96 ns	5.34 ns	0.64 ns	0.78 b	4.28 b	88.7 ns
messenger + 3(Ca sprays)	45.80	10.32	112.20	5.66 b	5.88	13.54	1.92	3.46	0.58	0.72 b	3.84 b	94.3
Ca(NO3)2 + 3(Ca sprays)	50.60	10.65	115.60	5.68 b	5.58	11.80	1.94	14.26	0.60	0.64 b	3.88 b	88.4
commercial + 3(Ca sprays)	53.60	12.12	127.80	8.30 a	6.50	13.68	2.20	4.20	0.74	1.92 a	8.56 a	92.0
P>F	0.3681	0.4137	0.7078	0.0521	0.3793	0.7139	0.5695	0.4269	0.1717	<.0001	0.0089	0.3461
$LSD_{(5\%)}$	12.618	2.6575	30.19	2.2375	1.2669	5.4868	0.4823	15.491	0.1561	0.4082	2.8421	7.8457

TABLE 9

Maturity indexing results of 'Golden Delicious' apple fruit at harvest (02/2010).

Treatment	ACID	TSS	FIRM	DIAM	MASS	1BACKCOL	RUSSET	STARCH
		%	kg	mm	g		%	%
control + 3(Ca sprays)	0.58 ns	12.9 ns	8.26 ns	63.06 ns	111.82 ns	1.56 ns	1.80 ns	21.10 b
messenger + 3(Ca sprays)	0.50	13.04	8.02	63.66	115.32	1.58	0.80	18.70 b
Ca(NO3)2 + 3(Ca sprays)	0.48	12.74	8.02	63.70	115.60	1.56	0.20	22.00 b
commercial + 3(Ca sprays)	0.46	12.54	7.88	64.44	119.62	1.58	0.00	34.80 a
P>F	0.384	0.584	0.392	0.628	0.570	0.927	0.192	0.007
$LSD_{(5\%)}$	0.197	0.630	0.466	2.251	11.742	0.092	1.832	8.631

1BACKCOL – fruit ground colour according to colour chart

TABLE 10

Maturity evaluation results of 'Golden Delicious' apple fruit after two months cold storage at 0.5˚C (06/2010).

Treatment	ACID	TSS	FIRM kg	DIAM mm	MASS g	1BACKCOL	2BP log	RUSSET log	3BP %	RUSSET %
control + 3(Ca sprays)	0.32 ns	13.82 a	5.52 a	61.37 ns	106.01 ns	3.66 b	-5.20 ns	-3.22 a	0.36	4.28
messenger + 3(Ca sprays)	0.28	13.64 a	5.57 a	60.25	101.97	3.96 a	-4.20	-3.44 a	1.86	4.24
Ca(NO3)2 + 3(Ca sprays)	0.31	13.70 a	5.50 a	60.68	105.00	3.77 b	-4.75	-3.84 a	1.4	2.2
commercial + 3(Ca sprays)	0.32	12.62 b	5.12 b	61.44	107.55	3.65 b	-4.81	-4.75 b	0.88	0.84
P>F	0.5483	0.0775	0.0279	0.757	0.863	0.007	0.500	0.009		
$LSD_{(5\%)}$	0.059	1.001	0.309	2.802	14.655	0.176	1.382	0.843		

1BACKCOL – fruit ground colour according to colour chart
2BP Logit = LOG((Bitter pit fruit + 0.5)/(Total no. fruit – Bitter pit fruit + 0.5))
3BP % - number of bitter pit fruit in the sample as percentage

TABLE 11

Actual costs of applying foliar products used in trial 1 and 2 as on January 2011.

Product	Cost/product	Product/tree	Cost/ tree	Cost/g (Ca)
Trial 1				
Calcimax™	R23.50/L	58 ml	R 1.16	R 0.20
Calflo™	R28.63/L	86 ml	R 2.46	R 0.24
Trial 2				
Calflo™	R28.63/L	37.6 ml	R 1.08	R 0.24
Calcinit™ (Commercial)	R44.2/kg	71.68/g	R 3.17	R 0.37

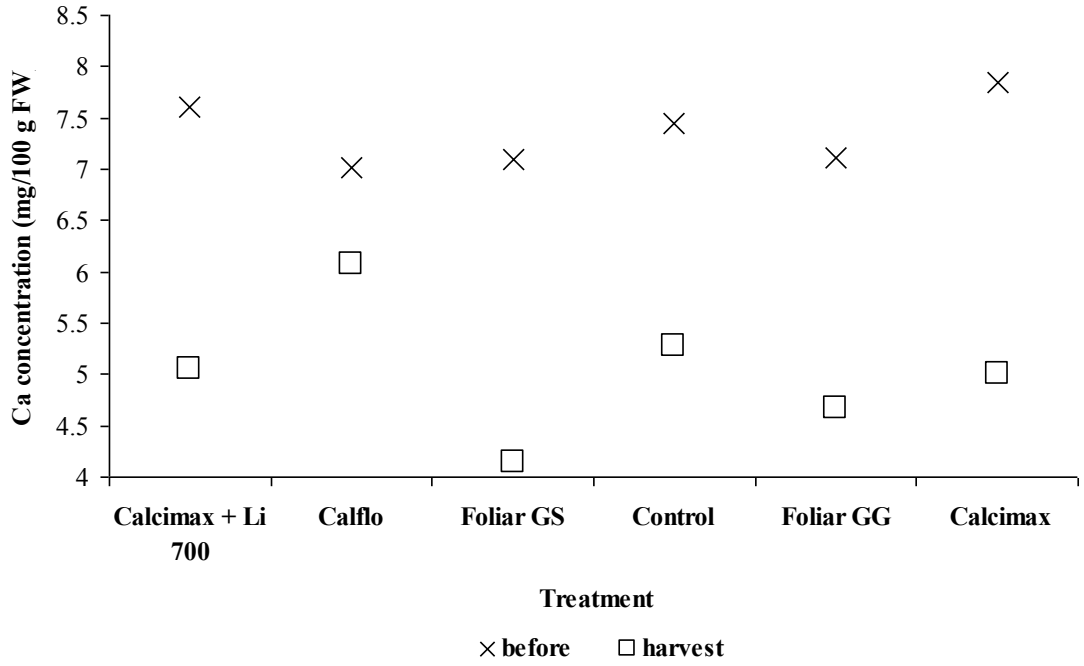

Fig. 1. Changes in 'Braeburn' apple fruit Ca concentration for the different treatments (before and after application) (2008/2009).

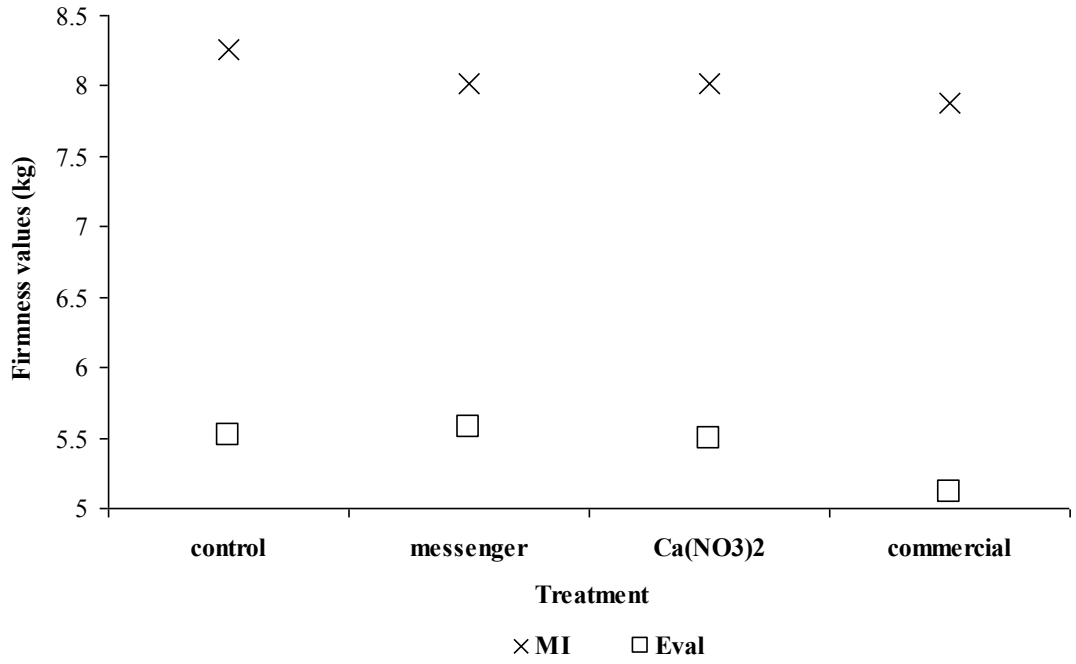

Fig. 2. Firmness values of 'Golden Delicious' apple fruit for the different treatments at harvest and after two months cold storage 2009/10.

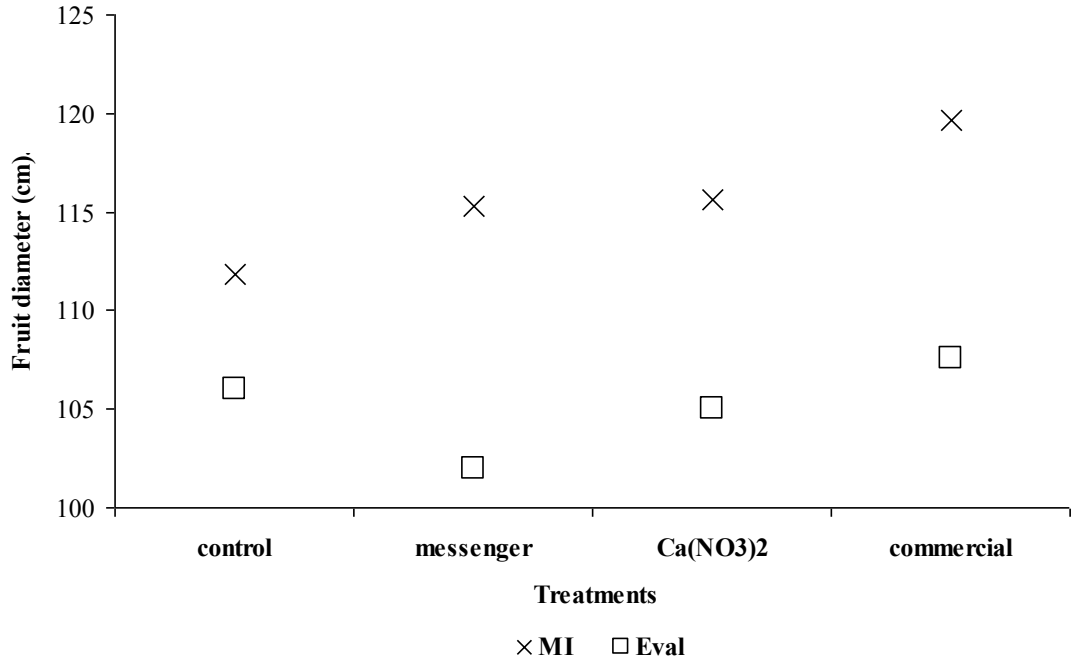

Fig. 3. Fruit diameter of 'Golden Delicious' apples for the different treatments at harvest and after two months cold storage 2009.

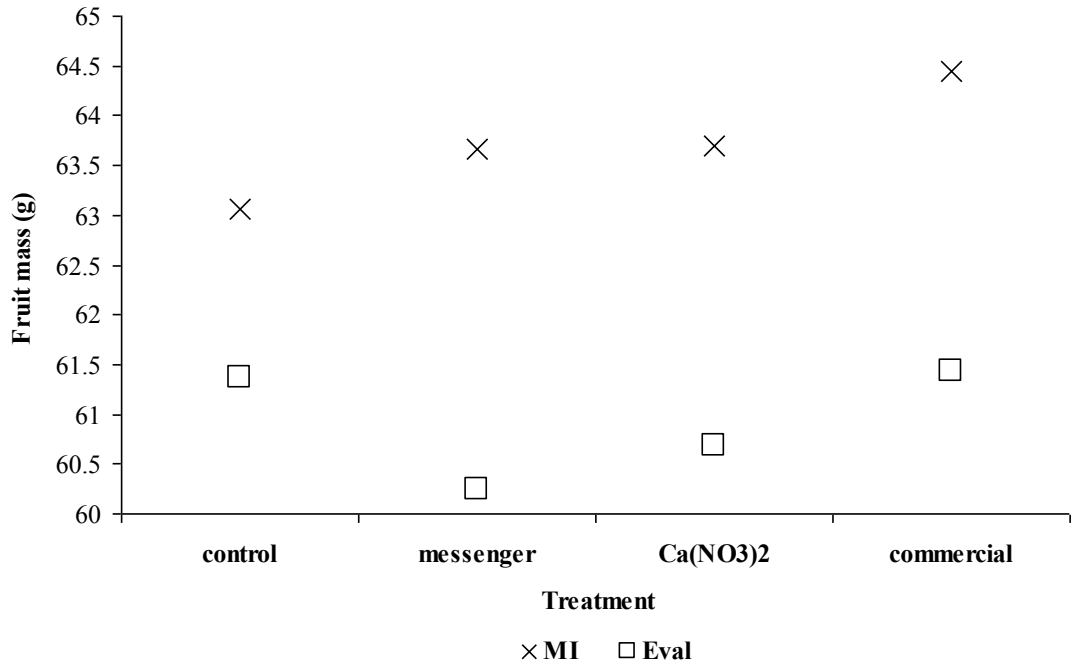

Fig. 4. Fruit mass of 'Golden Delicious' apples for the different treatments at harvest and after two months cold storage 2009.

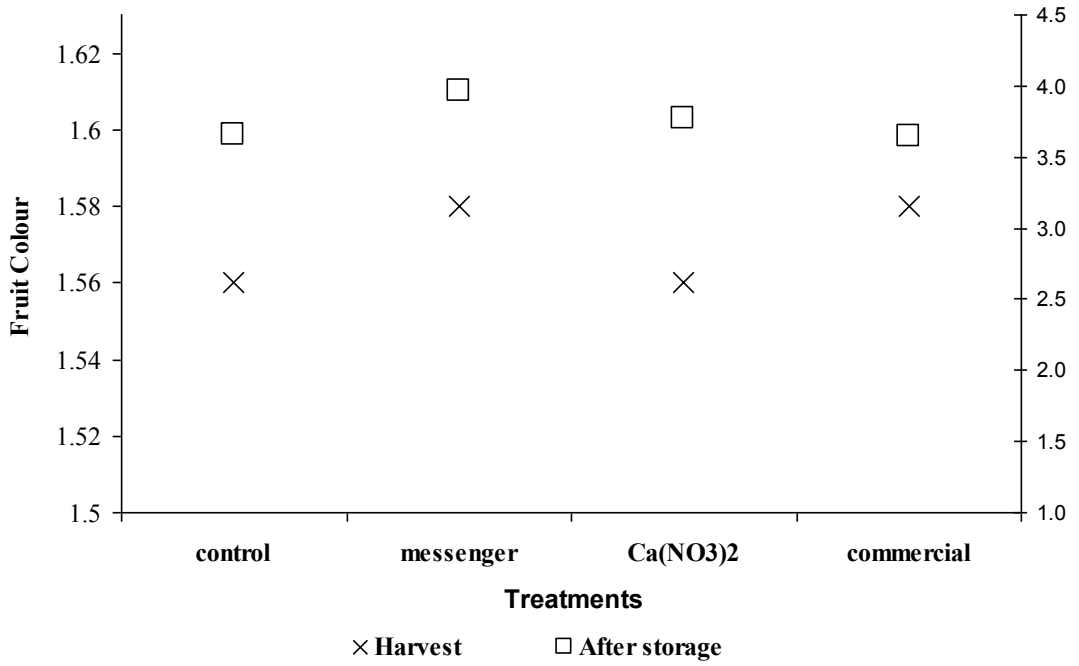

Fig. 5. Back ground colour of 'Golden Delicious' apples for the different treatments applied at harvest and after two months cold storage 2009

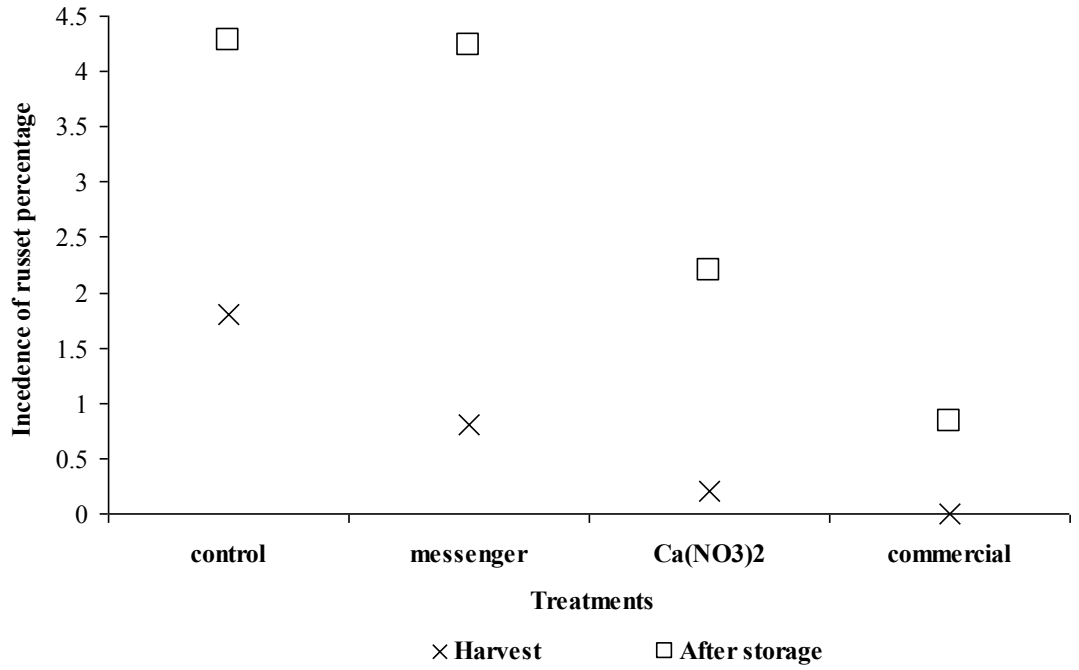

Fig. 6. Russet development in 'Golden Delicious' apples for the different treatments applied at harvest and after two months cold storage 2009.

Chapter 4: Paper 3

Mapping the distribution of calcium in apple tissue with proton-induced X-ray emission – after application of additional pre-harvest foliar or soil calcium

SUMMARY

The development of calcium (Ca) deficiencies in the apple fruit are ascribed to a lack in accumulation of Ca in specific areas of the fruit. Localized deficiencies therefore commonly occur in spite of sufficient levels of total fruit Ca. A trial was conducted during two consecutive seasons (2008/9, 2009/10) to evaluate the contribution of either foliar or soil applied Ca on the localization of Ca in "Braeburn" apple fruit. Ca ($Ca(NO_3)_2$) was applied either in the form of soil pellets (Tropicote™) at fruit set or after harvest, or as a series of weekly foliar sprays (Calflo™) between 21 and 70 days after full bloom. Additional treatments consisting of combinations of these were also applied. Elemental Ca mapping across the radius of the fruit was achieved via the use of micro-PIXE (a technique based on the use of particle induced x-ray emission). In all the treatments, Ca was found concentrated in the skin and core of the fruit, with the lowest values occurring in the outer cortex. At 80 dafb, Ca was highly associated with vascular bundles in an otherwise homogenous cortex. Fruit set soil applications of Ca consistently resulted in fruit with less Ca at 80 dafb when compared to the other treatments and did not contribute significantly to the new growth. It is therefore important to supply the tree with enough reserve Ca in order to ensure a sufficient Ca supply for future seasons. The prevalence of vascular bundles mostly determined the average Ca concentrations in the core, inner and outer cortex regions and emphasizes the importance of the functionality of vascular bundles throughout the season. Foliar Ca sprays were however sufficient in altering the distribution of Ca in the fruit and resulted in its increase in the outer cortex relative to the fruit's core.

Keywords: elemental mapping; PIXE; X-ray microanalysis, Ca deficiency; bitter pit; *Malus domestica*

Calcium (Ca) differs from other nutritional elements as most of it becomes incorporated in the foliage and permanent structures of the tree, with only small amounts being translocated to the fruit, and thus Ca-related deficiencies are a common appearance in apple orchards worldwide (Saure, 2002; 2005). Physiological disorders such as bitter pit and other quality defects arising from Ca deficiency, usually result in consignments from South Africa being rejected for the export market (Rousseau, 1972; Hanekom, 1975). This decreases the potential profit to the producer (Rousseau, 1972; Saure, 2005; Lötze & Theron, 2006). Although the total Ca content in the fruit is often sufficient for good quality, the distribution of Ca within the fruit tissues often leads to localised deficiencies in the fruit (Saure, 2005).

Ca is typically concentrated within the skin and core of the fruit (Ferguson & Watkins, 1983; Himelrick & McDuffie, 1983). From the core, Ca concentrations gradually decrease across the radius of the fruit, with the lowest values occurring in the outer cortex (Wilkinson & Perring, 1961). This is ascribed to a higher growth rate in the outer regions of the fruit as it expands throughout the season, causing a dilution in Ca concentration in the cortex (Saure, 2005). A decreasing number of vascular bundles towards the outer areas of the fruit may also contribute towards this distribution (Hanekom, 1975).

Soil applied Ca is absorbed through the root surface and then translocated to the fruit and leaves via the vascular system (White, 2001). Ca is exclusively transported apoplastically with water and other minerals in the xylem, and is highly immobile in the phloem (Bangerth, 1979; White, 2001). Ca reserves in the bark and wood can also supply fruit with Ca, and it has been shown

that these reserves can supply the total new growth with up to 25% of the required Ca (Terblanche *et al*., 1979; Ferguson & Turner, 1981; Himelrick & McDuffie, 1983). The xylem vessels in fruit are very rigid and inelastic (Drazeta *et al*., 2004). As the fruit expands and develops, these vessels tend to break down and become dysfunctional (Drazeta *et al*., 2004). This happens earlier in the season for cultivars like 'Braeburn' (45 days after full bloom), and partly explains its susceptibility to localised Ca deficiencies (Drazeta *et al*., 2004; Neilsen *et al*., 2005).

Foliar Ca sprays are applied as a standard practice to increase fruit Ca and reduce the incidence of bitter pit both internationally and in South Africa (De Villiërs and Hanekom, 1977; Terblanche *et al*., 1980; Ferguson & Watkins, 1989; Saure 2002; 2005; Lötze & Theron, 2007). Sufficient fruit Ca concentrations have been maintained by the application of six foliar sprays without any soil applied Ca (Lötze *et al*., 2008). Only the Ca that is applied directly onto the fruit surface will contribute toward its Ca status, as Ca is not remobilised from the leaves towards the rest of the tree in significant amounts (≤5%) (Hanekom, 1975; Schlegel & Schönherr, 2002; Saure, 2005).

Late season foliar sprays are known to be more effective in increasing the total Ca concentration of the fruit when compared to early season sprays (Neilsen & Neilsen, 2002; Schlegel & Schönherr, 2002; Neilsen *et al*., 2005). However, these sprays only penetrate the surface of the fruit by about five millimetres or less (Hanekom, 1975). Therefore, the outer regions of the fruit benefit most from foliar application of Ca and it is here where the symptoms of bitter pit are most expressed (Hanekom, 1975). Early season Ca sprays mainly penetrate through trichomes on the fruit surface, early in its developmental stages (Schlegel & Schönherr, 2002; Lötze & Theron, 2006). These sprays have been shown to be more effective in controlling bitter pit in

spite of a lower efficiency in increasing fruit Ca status (Neilsen & Neilsen, 2002; Schlegel & Schönherr, 2002; Neilsen *et al*., 2005; Lötze and Theron, 2007; Lötze *et al*., 2008).

Elemental mapping of apples using proton induced X-ray emission (PIXE) to describe the distribution of Ca within fruit has not been done before. Meyer *et al*. (1979) performed point analysis for determination of a concentration profile and have shown a rapid increase in the Ca concentration of pitted relative to non-pitted tissue. Only the pitted areas were used for this purpose. The concentration gradient of Ca in pitted fruit has been established by Meyer *et al*. (1982) in an attempt to quantify the optimum combination of nutritional elements to avoid the development of bitter pit. Ca was found to rapidly increase towards the core of the fruit and was ascribed to a higher amount of vascular bundles in this area (Meyer *et al*., 1982). PIXE have thus been proven to be a suitable method in undertaking nutritional studies in apples with the main advantage being its high detection sensitivity (Meyer *et al*., 1982).

Commercial Ca supplements to apple trees in South Africa are either applied as soil applications after set and after harvest or a series of foliar sprays after set. Thus the Ca supply from the soil or from the reserve pool of the tree enters the fruit through the vascular bundles (xylem vessels) (Saure, 2005). In contrast, the point of entrance of foliar applied Ca, is the fruit surface (Harker & Ferguson, 1991; Trentham *et al*., 2008).

The aim of this trial is firstly, to establish the distribution pattern of Ca in the fruit resulting from the different Ca application strategies and secondly, to determine Ca concentrations in the various tissues (peel and cortex) for each treatment, using micro-PIXE.

MATERIALS AND METHODS

Fruits were selected from mature, full bearing 'Braeburn' apple trees, planted on rootstock M793 at a tree spacing of 4 m x 1.5 m on a commercial farm, Eriskay, in the Vyeboom area. An existing trial (Paper 2), where different treatments (combinations of either soil- and/or foliar applied Ca) were performed, was used for sampling.

Three fruits per treatment were selected during two consecutive seasons (2008/9 and 2009/10) at 80dafb (Dec 2009 and 2010) and at harvest (Mar 2009 and 2010) for microanalyses at iThemba LABS, Somerset West, South Africa. Samples were selected from spurs on two-year old wood to reduce variability. Due to an extended period of time needed to analyse every sample and a limitation of beam time allocated to the project, only three replicates per treatment (5) were used (15 samples per season). For each replicate, two fruits were sampled, and two diagonal sections per fruit were utilized.

Additional fruit were also selected from mature, full bearing 'Golden Delicious' apples, planted on M793 on a commercial farm, Queen Anne, also in the Vyeboom area. An existing trial (Paper 2), where different foliar products were applied, was used for sampling. Only four fruits in total were used for this purpose (one fruit per treatment).

A razor blade was used to cut a thin, cross-sectional apple slice diagonally across the radius of each fruit as soon as possible after sampling (Figure 7). At 80 dafb, the radius of the apples was approximately 14 mm, and at harvest, approximately 26 mm. Specimens were preserved by rapid plunge cryofixation in liquid propane cooled with liquid nitrogen, using a Leica EM CFC Cryoworkstation. A Leica EM CFD Cryosorption Freeze Dryer was used to lyophilise the specimens which were then stored for further X-ray microanalysis in a desiccator with a silica-

gel. Specimens were then mounted between the two Formvar films (0.5% Formvar) spread on aluminum frames, using Araldite glue. The front Formvar film (facing the proton beam) was coated with a fine carbon layer to prevent charge build up during measurements. The aluminum frames containing the desired samples were attached to a motor-driven sample ladder mounted inside the experimental chamber of the nuclear microprobe, in vacuum of the order of 10^{-5} mbar.

X-ray microanalyses were performed using the nuclear microprobe at the Materials Research Department of iThemba LABS. A proton beam of 3 MeV energy, provided by the 6 MV single-ended Van de Graaff accelerator, was focused to a 5 x 5 μm^2 spot and repetitively scanned over specimens using square scan patterns (scan size 2.6 mm x 2.6 mm; 128 x 128 pixels; dwell time per pixel 10 ms). The accumulated charge for most scans was 300 nC (first few scans were done for 2 μC and 1 μC). The proton current was kept below 300 pA to minimize specimen beam damage. Particle-induced X-ray Emission (PIXE) and proton backscattering (BS) were used simultaneously. PIXE spectra were registered in the energy-dispersive mode, using a Si(Li) detector (active area: 30 mm^2; resolution: ca. 160 eV for Mn Kα line) positioned at a take-off angle of 135° and a working distance of 25 mm. The x-ray energy range was set between 1 and 36 keV and an external absorber (125 μm Be) was positioned between the detector and the specimen to stop backscattered protons. BS spectra were recorded with an annular Si surface barrier detector (100 μm thick) positioned at an average angle of 176°. Data were acquired in the event-by-event mode. The normalization of results was done using the integrated beam charge collected from the insulated specimen holder. A more detailed description of the nuclear microprobe setup at iThemba LABS can be found in Prozesky *et al*. (1995) and Przybylowicz *et al*. (1999, 2001, 2005).

Each specimen (one slice per treatment), from fruits sampled at 80 dafb (2009/10) and harvest (2008/9), was scanned repetitively over its whole radius. Data evaluation was performed using GeoPIXE II software (Ryan, 2000). The composition of the major, light elements in the samples (the "matrix" composition) was established from the analysis of proton backscattering spectra using a RUMP simulation package (Doolittle, 1986) with the non-Rutherford cross-sections for C, O and N. It was expressed in atomic ratios as $C_{17}H_{67}O_{15}K_{0.19}$. PIXE spectra from scanned areas were fitted using a full nonlinear de-convolution procedure (Ryan *et al.*, 1990a, b). The errors of analysis were extracted from the error matrix generated in the fit (Ryan *et al*., 1990a), whereas the minimum detection limits were calculated using the Currie formula (Currie, 1968). An average Ca concentration was calculated for each individual scan. The information on the average concentrations from scanned areas was complemented by quantitative elemental images generated using the *Dynamic Analysis* method (Ryan & Jamieson, 1993; Ryan *et al*., 1995). Elemental images were mainly used to check the homogeneity of the distribution of elements within the scanned areas. An average of 12 scans was necessary to cover the distance from peel to core in each sample and these were integrated to show the elemental distribution over the length of each sample. A linear traverse was fitted onto each group of the integrated scans to yield a continuous graph of Ca concentration over the length of sample analyzed. In addition, the average Ca concentration for three specimens per treatment was determined for three sections: core, middle and outer cortex. Although the main attention was paid to the concentration and distribution of Ca, the experimental conditions allowed for the detection of many other elements as well (Al, Si, P, S, Cl, K, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn).

RESULTS AND DISCUSSION

Ca concentrations are the highest in the peel compared to the other tissues (Figures 1-5). Ca concentrations are also high in the core, compared to the middle and outside of the fruit cortex, although these values are typically lower when compared to the peel. These observations are in agreement with existing literature regarding the typical distribution of Ca from skin to core (Wilkinson & Perring, 1961; Ferguson & Watkins, 1983; Himelrick & McDuffie, 1983). It seems that the high concentrations of Ca in the core of the fruit are due to a higher prevalence of vascular bundles toward the inside of the fruit (MacArthur and Wetmore, 1939). This leads to an enrichment of Ca in an otherwise a homogenous distribution of flesh Ca (Figures 1-5). This supports the findings of Meyer *et al*. (1982).

Fruit sampled 80 dafb

At 80 dafb in 2008/9, the distribution of Ca, obtained from calculating the average Ca concentration in the core, middle- and outer cortex of three replicate specimens, was similar for all treatments in all three regions of the apple. The only exception being the consistently lower concentrations in the treatment where only fruit set Ca was applied (Figure 8). Similarly, at 80 dafb in 2009/10, Ca concentrations of fruits that received only the fruit set soil Ca application were the lowest in the core, middle- and outer cortex (Figure 9). The Ca concentration in the peel was also the lowest for this treatment at 80 dafb, both in 2008/9 and 2009/10, compared to the other treatments (Figure 10). Bulk mineral analyses of similar samples (Paper 2) showed no significant differences between the treatments in 2008/9. However, in 2009/10, Ca concentrations of the fruit form the fruit set soil Ca treatment only, were significantly lower than the treatments where i) Ca was applied as a foliar spray and ii) soil Ca was applied both at fruit set and post harvest (Wilsdorf *et al*., 2010; Table IV). Average Ca concentrations in 2009/10 were the highest in the core, middle- and outer cortex for the treatment where post harvest and

fruit set soil Ca applications were made, compared to other treatments (Figure 9). When Ca concentration of this treatment was mapped continuously from core to peel for one specimen, the highest Ca concentration occurred towards the core, due to enriched areas of Ca associated with vascular bundles (Figure 3). In contrast, both treatments where foliar Ca was applied had a higher average Ca concentration in the outer cortex, compared to the core (Table I). When considering continuous Ca mapping of individual specimen in 2008/9 and 2009/10, the effect of a lower average Ca concentration in the core (relative between treatments) was often masked if vascular bundles were present in the section analysed (Figures 2,3,8 & 9). However, when considering the outer cortex, just beneath the peel of the fruit, where vascular bundles were not as prevalent, the treatment where fruit set soil Ca was applied, seems to have the lowest values in this region with Ca concentrations decreasing to 0 ppm in various locations in 2009/10 (Figure 4). According to literature, this is the region where localised Ca deficiency usually develops and symptoms of bitter pit occur (Rosseau, 1972; Hanekom, 1975, Saure 2005).

When additional fruit were sampled, in a separate trial, for comparison of the penetration efficiency of different foliar products, Ca concentrations in the outer cortex did not vary much between treatments (Table II). All treatments had higher Ca concentrations in the outer cortex when compared to the control (Table II). The treatment where Calflo^{TM} was applied had a higher Ca concentration than the commercial treatment and the application of Messenger^{TM} resulted in the highest Ca concentration (Table II).

Fruits sampled at harvest

Average Ca values from the core to the outer cortex were much lower at harvest (150 – 350 ppm) compared to fruits sampled 80 dafb (200 – 600 ppm) (Figures 8, 9 & 11). This is in agreement with the Saure (2005) who reported that Ca concentrations decrease as fruits expand towards harvest. Where continuous mapping of individual specimen was done, the presence and contribution of vascular bundles on the Ca concentration in the core was substantial for fruits at harvest (Figure 5), again confirming the importance of xylem functionality (Drazeta *et al*., 2004). The average Ca distribution for specimen at harvest 2008/9 also differed from those sampled at 80 dafb (2008/9). At 80 dafb, the outer cortex Ca concentration was either higher, or of similar magnitude, compared to the middle cortex concentrations for all the treatments (Figure 8 & 9). At harvest, average Ca concentrations in the outer cortex region were always lower than in the middle cortex, and the decrease in Ca concentration form the core to the outer cortex was more uniform (Figure 11). The treatment where only foliar sprays were applied, had the highest average Ca concentrations in the core, middle- and outer cortex, compared to the other treatments at harvest of 2008/9 (Figure 11). This was also the case for continuous mapping of individual specimen, especially with the higher amounts of Ca in the foliar treatment than in the outer cortex (Figure 6). Average Ca concentrations in the outer cortex differed the least from its core concentrations in the foliar spray treatment, compared to the other treatments (Table I). Both treatments where foliar sprays were applied had the highest skin concentrations at harvest of 2008/9 (Figure 12). No significant differences in Ca concentration were found from bulk mineral analyses of fruits at harvest in 2008/9 (Wilsdorf *et al*., 2010; Table V). Only one fruit per treatment was analysed for elemental mapping at harvest of 2009/10 due to a time constraint, and thus no average Ca values are available, so no definite conclusions can be made from this result which only presents one data point. Nevertheless, average regional Ca concentrations were higher from PIXE analyses when compared to its corresponding mineral analyses results (Paper

2). This is partly because the total amounts of Ca are measured with PIXE analyses versus only soluble Ca measured through mineral analyses. Mineral analyses would therefore be a better indicator of total fruit Ca deficiency compared to average PIXE analyses, as only the water soluble Ca is regarded to be physiologically active and relates to the incidence of bitter pit (Pavicic *et al*., 2004). Although PIXE analyses are interpreted in terms of the total number of Ca atoms, in this trial it provides a better indication regarding the effect of different Ca applications methods on the distribution of Ca in fruit tissue. Throughout this trial period, the incidence of bitter pit was very low (0-2.99%) and no significant differences occurred between treatments (Wilsdorf *et al*., 2010; Tables XIV, XV & XVI) and could thus be linked directly to either the Ca concentration *per se*, or the distribution of Ca in the fruit tissue.

Where fruits were sampled at the additional site, all treatments that received extra Ca sprays increased the outer cortex Ca concentration. At the stage of sampling at 80 dafb, six Calflo^{TM} and 4 commercial sprays were applied (Wilsdorf, 2010). Higher outer cortex Ca concentrations resulted from applying additional Ca sprays (Table II). Messenger^{TM} had the highest outer cortex Ca at 80 dafb, however this treatment resulted in the lowest core Ca concentration.

CONCLUSION

Ca is mostly concentrated in the peel and core of the fruit, with lower concentrations in the middle to outer cortex. Ca concentrations in the core of individual fruits are highly influenced by the prevalence of vascular bundles in this region as shown via PIXE mapping results at 80 dafb. These results indicate that soil applied Ca at fruit set does not contribute significantly towards the Ca concentration of the apple fruit from the present season. It would therefore be more important to supply the reserves of the tree with Ca by post harvest soil applications of the previous season. The prevalence of vascular bundles, and the amounts of Ca that it delivers to the fruit, mostly determines the total Ca concentration throughout the whole of the fruit (core, middle and outer cortex). Early disintegration of xylem vessels may thus play a pivotal role in terms of the total amount of Ca transported to the fruit, the source of the Ca, as well as the time of transport throughout the season. Nonetheless, the application of foliar Ca increases the Ca concentration in the outer cortex relative to its core, where localised deficiencies usually develop, and can still be an important means to increase the Ca concentration of the current season's fruit.

Currently, anatomical studies of the same specimen are also undertaken to determine whether the Ca distribution directly affects the cell wall structure visibly and if this varies between the different treatments, but these results will only be presented in future.

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TABLE I

Difference in average Ca concentration between the core and outer flesh of 'Braeburn' apples 80 days after full bloom 2009/10 (Jan 2010).

Treatment	Avg core Ca - Avg outer cortex Ca (ppm)	
	80 dafb 2009/10 (Jan 2010)	Harvest2008/9 (Mar 2009)
Soil application after harvest	-51.7	146.0
Soil applications after harvest & after fruit set	65.2	97.6
Soil application after harvest and foliar sprays after fruit set	-30.8	115.8
Foliar sprays after fruit set	-0.8	30.0
Soil application at fruit set	59.3	80.0

TABLE II

Ca concentration per 2mm scan across the radius of 'Golden Delicious' apples 80 days after full bloom 2009/10 (Jan 2010).

Treatment	Core	Inner cortex			Outer cortex			Skin
Control + 3(Ca sprays)	703	634	380	335	266	263	351	1765
Messenger™ + 3(Ca sprays)	X	368	457	436	301	343	408	2087
Calflo™ + 3(Ca sprays)	779	620	602	372	401	332	340	1329
Commercial + 3(Ca sprays)	586	518	347	322	293	303	360	2103

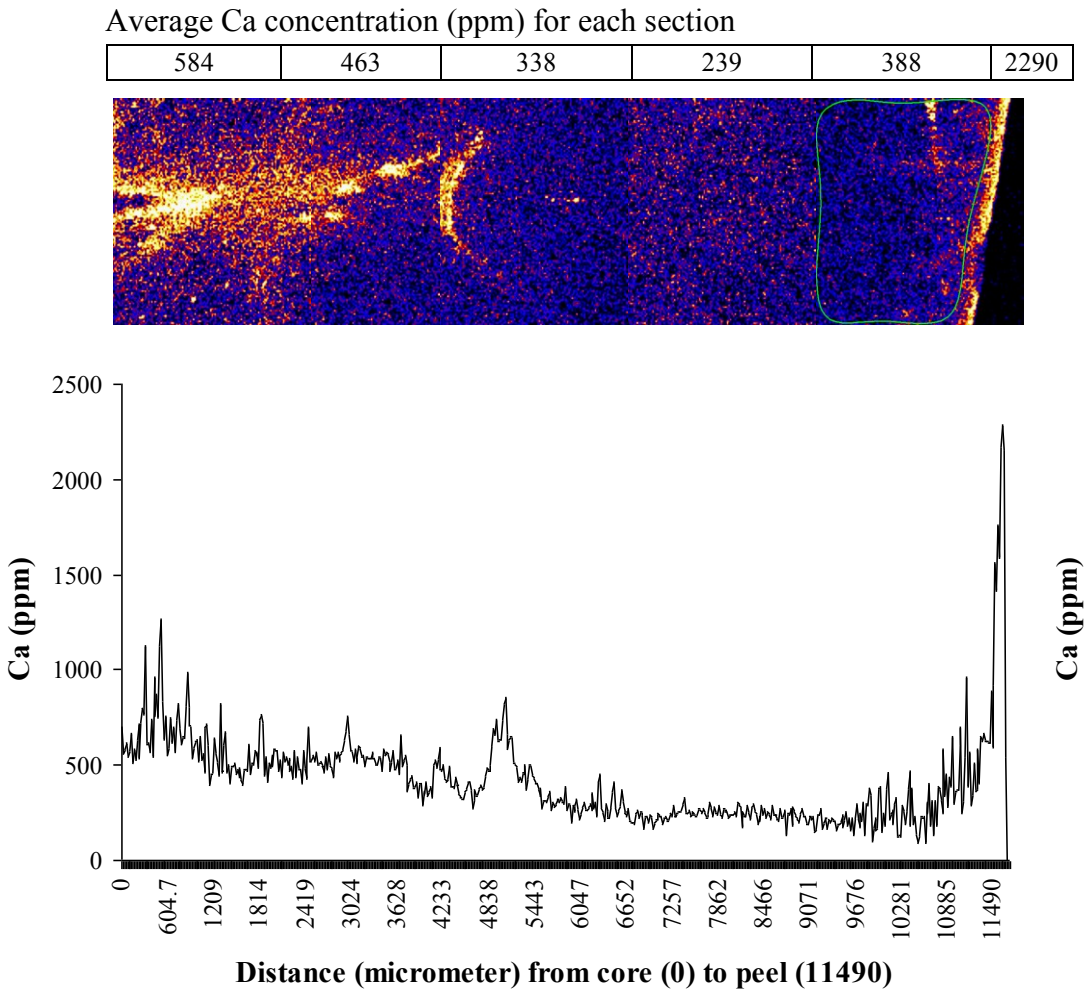

FIG. 1

Calcium map of a 'Braeburn' apple specimen of the post harvest soil $Ca(NO_3)_2$ treatment, sampled 80 dafb (2009/01), displaying 1) the average Ca concentration (ppm) for each section (peel concentration also included) and 2) a continuous plot of Ca concentrations form core to peel.

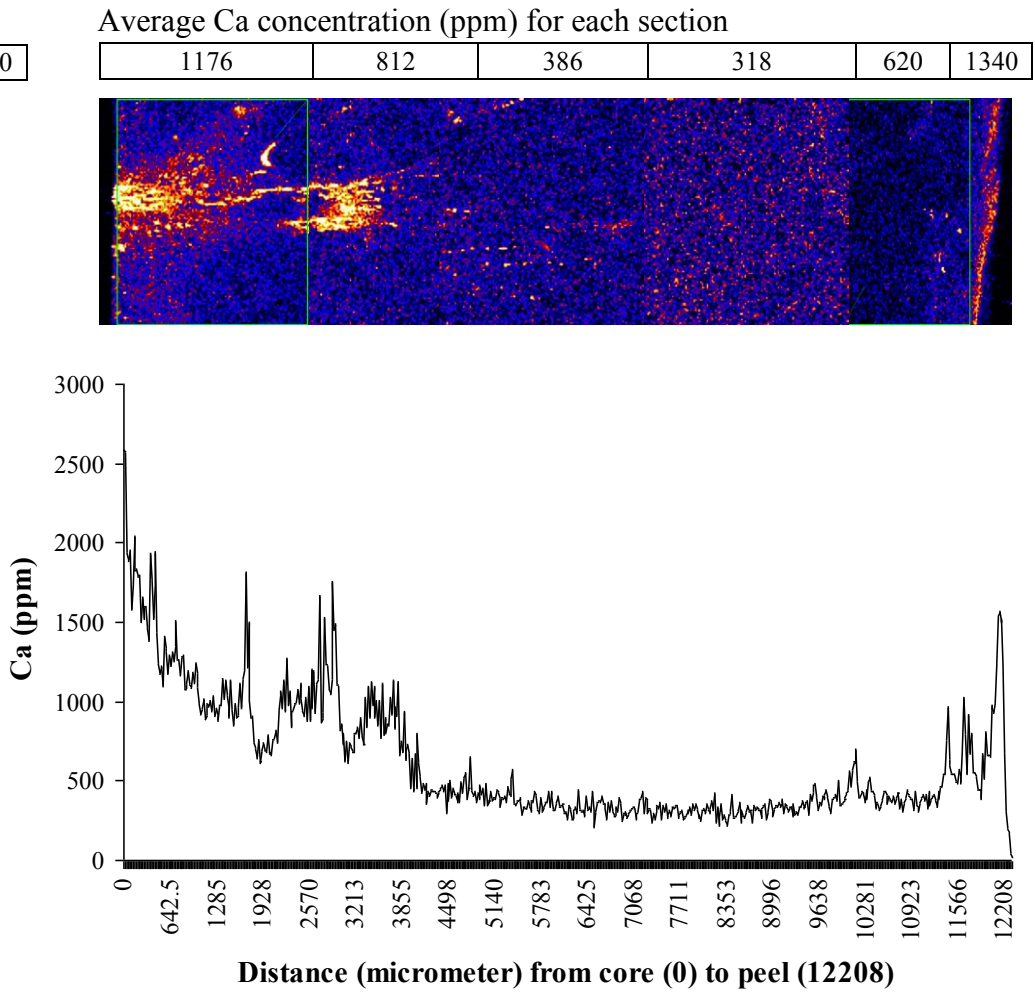

FIG. 2

Calcium map of a 'Braeburn' apple specimen of the post harvest soil $Ca(NO_3)_2$ and a fruits set foliar $Ca(NO_3)_2$ treatment, sampled 80 dafb (2009/01), displaying 1) the average Ca concentration (ppm) for each section (peel concentration also included) and 2) a continuous plot of the Ca concentrations form core to peel.

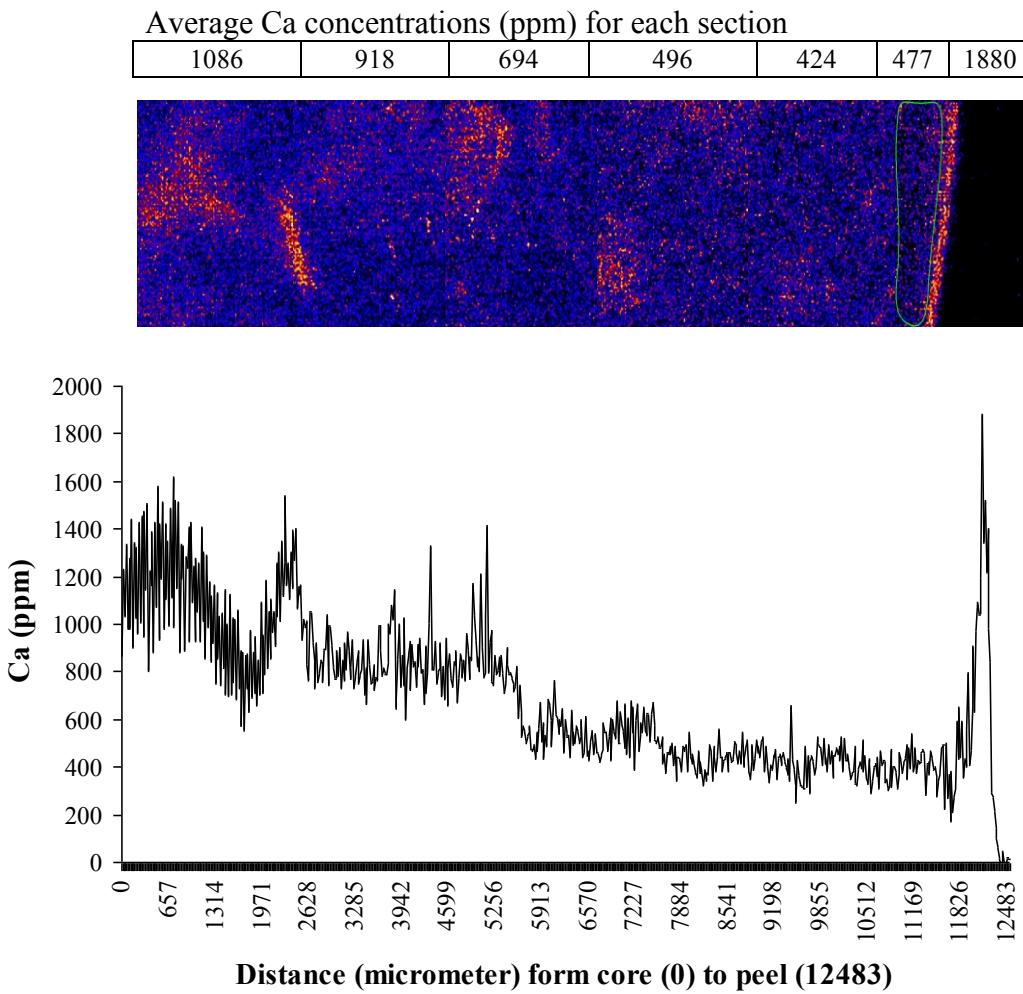

FIG. 3

Calcium map of a 'Braeburn' apple specimen of the fruit set and post harvest soil $Ca(NO_3)_2$ treatment, sampled 80 dafb (2010/01), displaying 1) the average Ca concentration (ppm) for each section (peel concentration also included) and 2) a continuous plot of Ca concentrations form core to peel.

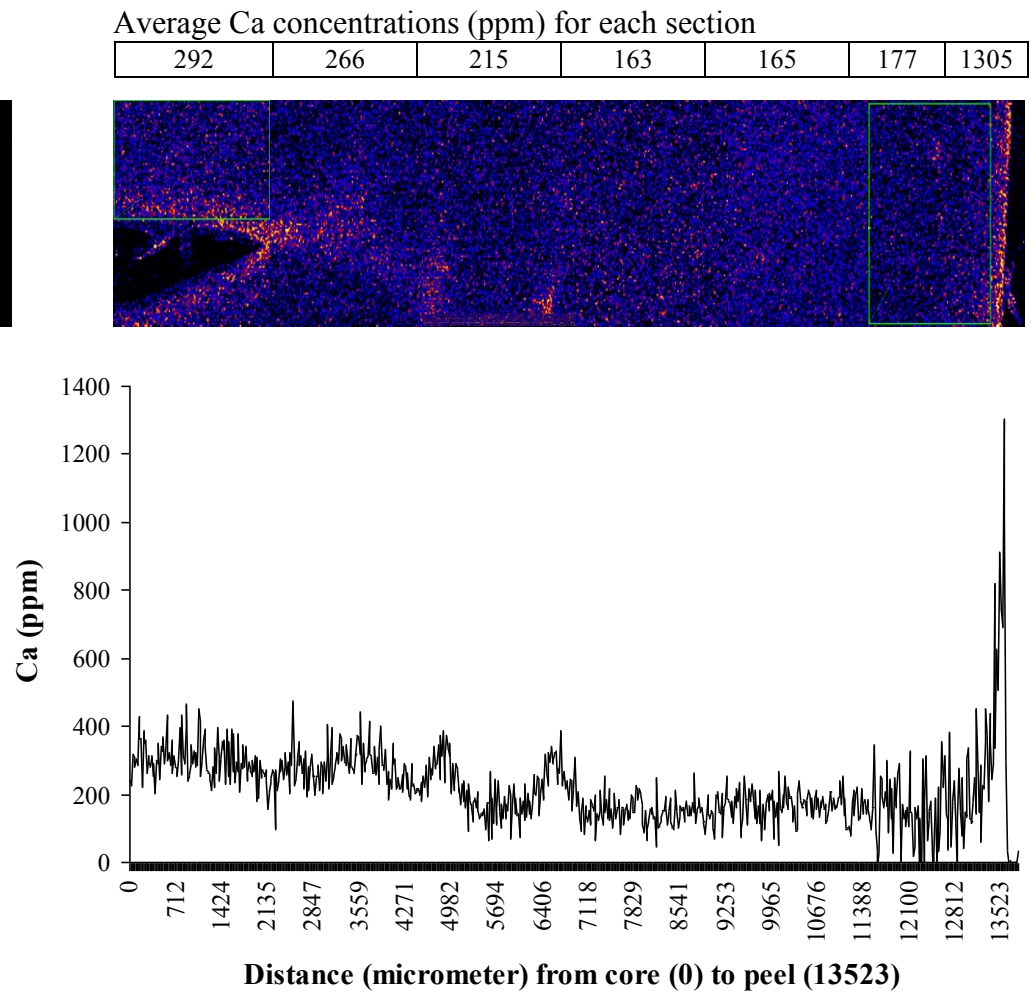

FIG. 4

Calcium map of a 'Braeburn' apple specimen of the fruit set soil $Ca(NO_3)_2$ treatment, sampled 80 dafb (2010/01), displaying 1) the average Ca concentration (ppm) for each section (peel concentration also included) and 2) a continuous plot of Ca concentrations form core to peel.

Average Ca concentrations (ppm) for each section

337	281	239	247	170	135

219	196	174	206	223	181	2750

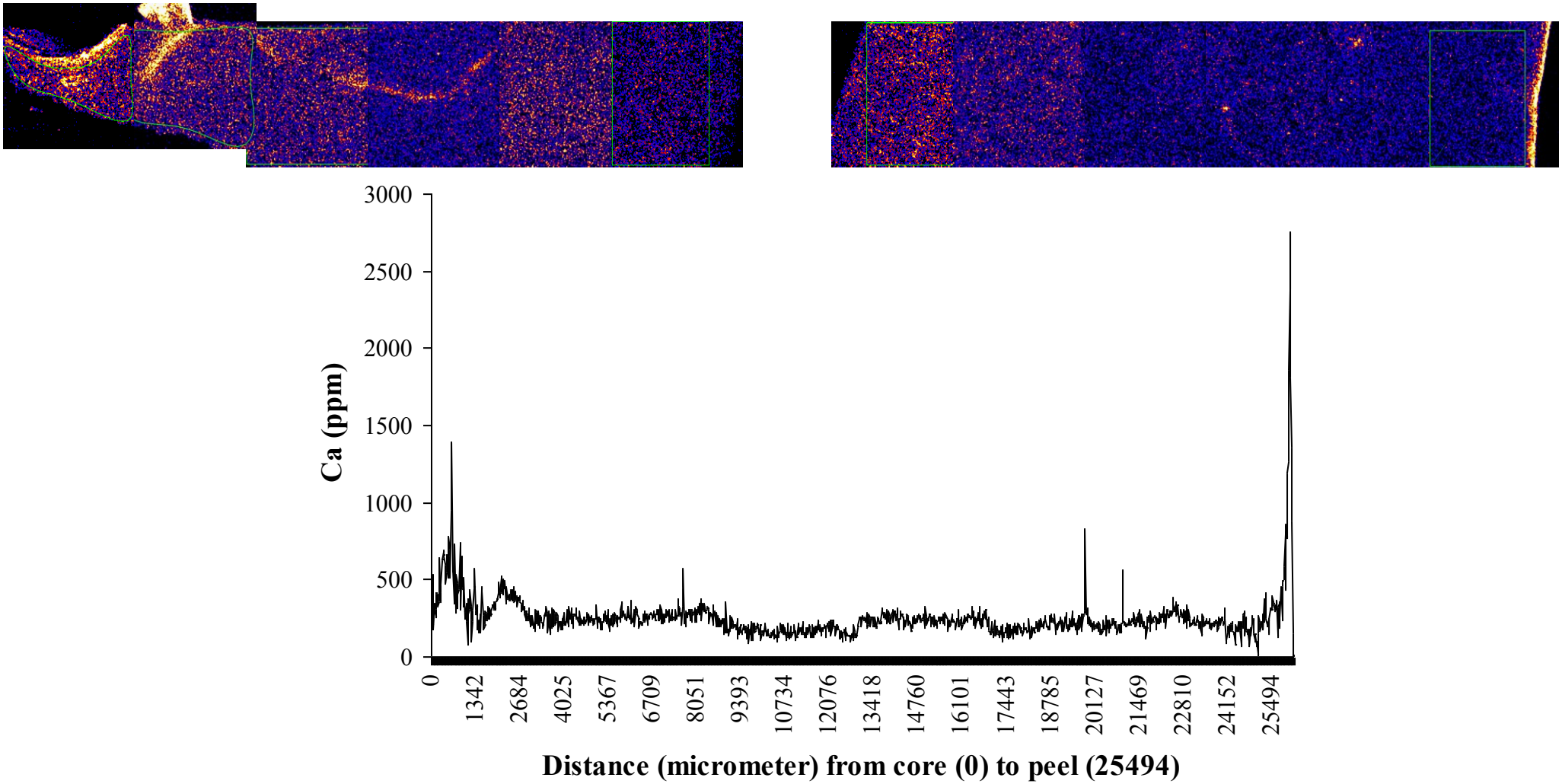

FIG. 5

Calcium map of a 'Braeburn' apple specimen of the fruit set soil $Ca(NO_3)_2$ treatment, sampled at harvest (2009/03), displaying 1) the average Ca concentration (ppm) for each section (peel concentration also included) and 2) a continuous plot of Ca concentrations form core to peel.

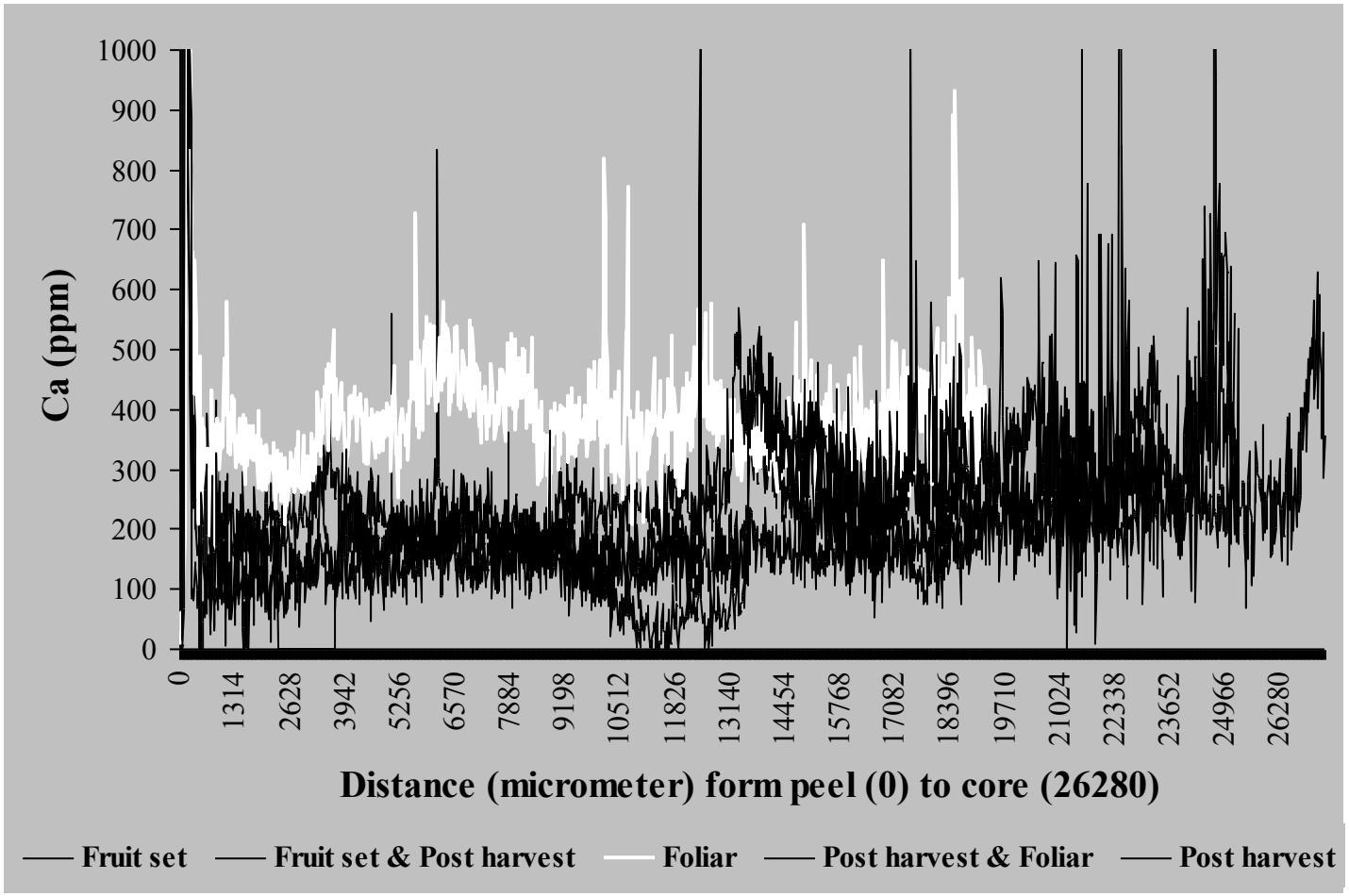

FIG. 6

Continuous Ca gradient of 'Braeburn' apple specimen (one from each treatment), sampled at harvest (2009), displaying the Ca concentrations form peel to core (foliar treatment indicated in white).

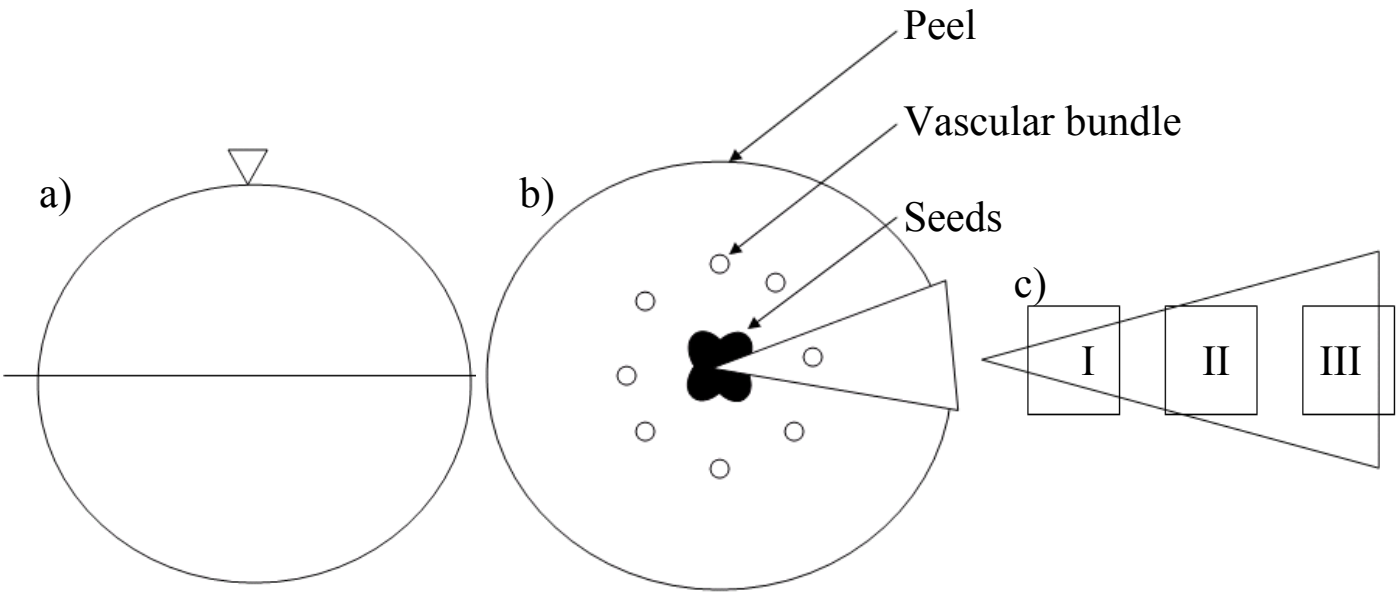

FIG 7

A diagram explaining the procedure of how specimen were sampled showing: a) the whole fruit cut diagonally, b) a diagonal section sampled across the radius of the fruit, and c) average Ca concentrations calculated from the I) core, II) inner cortex and III) outer cortex of the specimen.

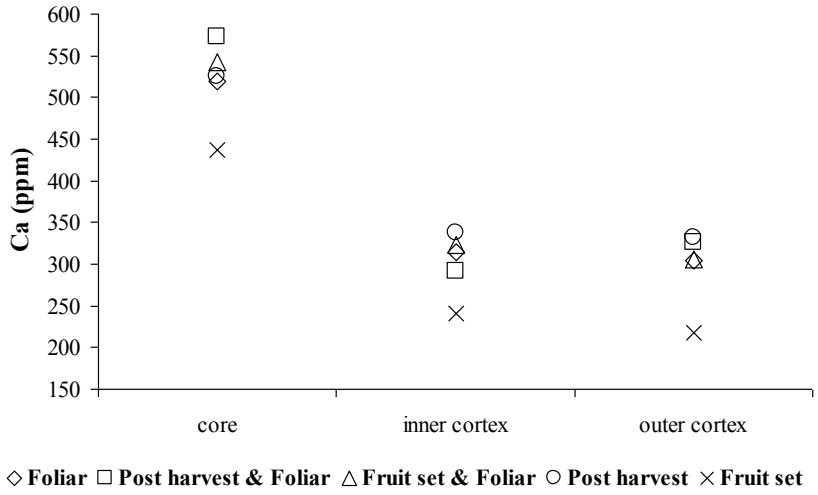

FIG. 8

Average Ca concentration in the core and cortex of 'Braeburn' apples according to treatment, sampled at 80 days after full bloom (Jan 2009).

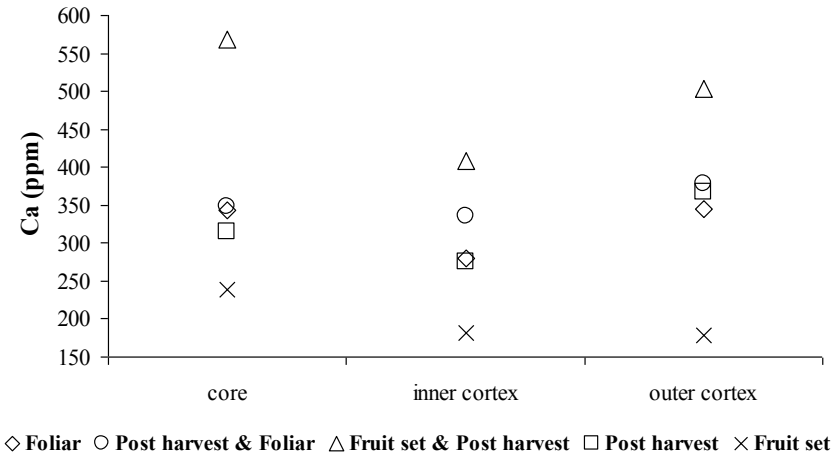

FIG. 9

Average Ca concentration in the core and cortex of 'Braeburn' apples according to treatment, sampled at 80 days after full bloom (Jan 2010).

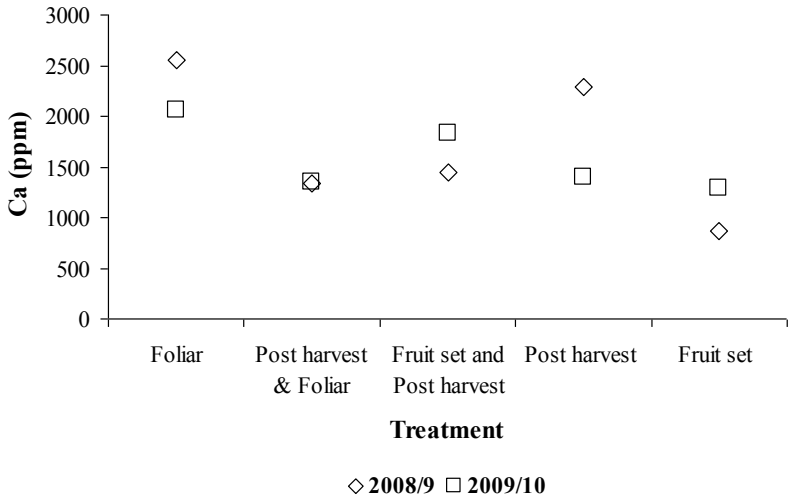

FIG. 10

Peel Ca concentration of 'Braeburn' apples according to each treatment, sampled at 80 days after full bloom (2008/9 & 2009/10).

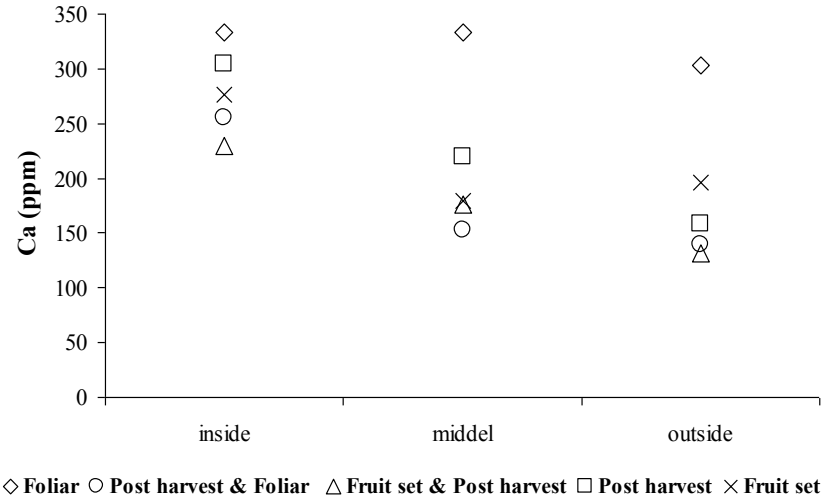

FIG. 11

Average Ca concentration in the flesh of 'Braeburn' apples, according to treatment, sampled harvest (2009/03).

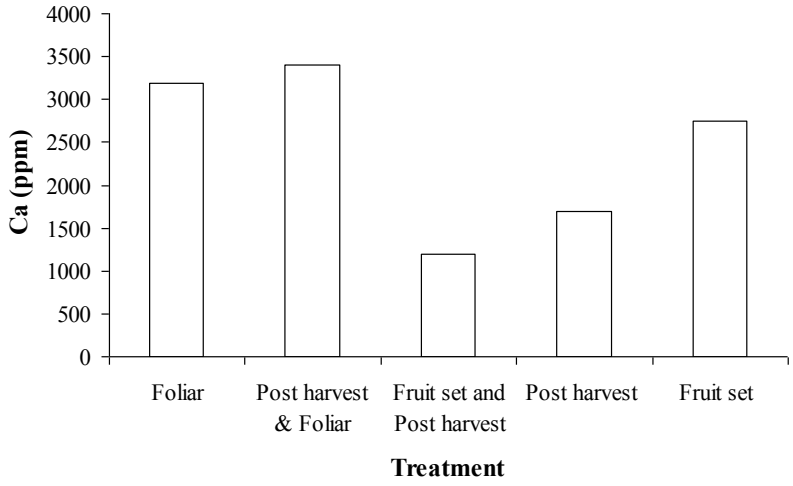

FIG. 12

Peel Ca concentration of 'Braeburn' apples according to treatment, sampled at harvest (2009/03).

General Discussion and Conclusions

Two trials were conducted in the Vyeboom area, close to Villiersdorp. In the first trial, $Ca(NO_3)_2$ was applied as soil applications after fruit set (end October to November) or after harvest (March to April). A series of six weekly foliar sprays were also applied from approximately 21 dafb. These were either combined or applied independently to comprise five different Ca fertilization strategies. This was applied to 'Braeburn' trees to evaluate the effect of the different application strategies on fruit Ca concentration. By using PIXE analyses, the effect of application strategy on fruit Ca distribution could also be investigated from core to peel. A second trial was conducted on 'Golden Delicious' apples. Here, the efficiency of different foliar Ca formulations in increasing total fruit Ca concentration was evaluated. The effect of all treatments on fruit quality after storage was also determined in these investigations.

According to various authors, bitter pit initiation occurs in the early part of the season and it is during this period that Ca supply to the fruit is critical (Neilsen and Neilsen, 2002; Schlegel and Schönherr, 2002; Neilsen *et al*., 2005; Lötze and Theron, 2007; Lötze *et al*., 2008). In the 'Braeburn' trial, early season foliar sprays of $Ca(NO_3)_2$ (applied weekly from 28 – 58 dafb) resulted in fruits with higher soluble Ca concentrations at 80 dafb (January 2010), compared to soil applications.

Active root growth was shown to be essential for effective Ca uptake from the soil (Bangerth, 1979; White, 2001). In this study, pre-harvest soil Ca applications at four to six weeks after full bloom had no significant effect on fruit Ca concentration. However, if applied when active root tips are visible (end November 2009) fruit Ca concentration at 80 dafb was significantly different between treatments. This emphasizes the importance of visual inspection of the root zone for active root tips before applying soil Ca.

Unexpectedly, although root growth was monitored before application, pre-harvest soil Ca did not contribute toward current season's fruit Ca concentration. Application of pre-harvest soil Ca (approximately four weeks after full bloom) resulted in fruits with the lowest Ca concentrations (March 2010) - significantly lower than the treatments where i) only foliar applications and ii) foliar applications together with post harvest soil Ca, were applied.

A possible explanation to this may be the anatomy of the fruit peduncle. In some apple cultivars, including 'Braeburn', disintegration of xylem vessels can occur from 40 dafb (Drazeta *et al*., 2004). If pre-harvest Ca is taken up by the roots after this stage, this Ca can thus not reach the fruit in significant quantities. Therefore soil applied Ca after pre-harvest should rather be regarded as enhancing the Ca reserves status of the tree. This will also play a significant role in supplying Ca to developing fruits for the subsequent season, when taken into account that most of the Ca supplied for initial fruit growth is relocated from the tree reserves as Ca is not yet absorbed from the root system (Saure, 2004). Ca reserves have been shown to contribute up to 25% of the Ca found in new growth (Terblanche *et al*., 1979; Himelrick and McDuffy, 1983). These factors, together with the fact that Ca transport will be favored to higher transpiring organs such as developing shoots and leaves, must be taken under careful consideration when applying pre-harvest soil Ca to cultivars such as 'Braeburn' that are susceptible to Ca deficiencies.

Ca related quality disorders such as bitter pit commonly exist in orchards with adequate Ca concentrations in the fruit at harvest. More specifically for bitter pit, these symptoms are typically expressed in the outer cortex of the fruit. It is in these outer cortex regions that localized Ca deficiencies exist in spite of adequate amounts of total Ca. Bulk mineral analyses would therefore have limitations regarding the prediction of possible development of deficiency

disorders in localized areas, as it does not supply any information as to the exact location of Ca in the fruit. For this reason, the first aim of the trial was to establish how different fertilization mechanisms could alter the distribution of Ca in the fruit, and then to quantify the effect of the change in distribution on fruit quality. In order to do this, apple tissue samples were analyzed using PIXE analyses. Via a microprobe, Ca maps were created across the radius of the fruit and the distribution of Ca for each treatment, established. Ca was found to be mainly concentrated in the core and skin of the fruit, with the lowest values occurring in the outer cortex, both at 80 dafb and at harvest, confirming previous findings (Wilkinson and Perring, 1961). PIXE results consistently indicated that fruits with the lowest Ca concentration resulted from trees receiving only pre- harvest soil Ca. This is in agreement with results in this study that derived from bulk mineral analyses. Furthermore, Ca concentrations also decreased to extremely low values, under the detection limit in some cases, in the outer cortex region of this treatment. Surprisingly, the prevalence and functionality of vascular bundles in the fruit cortex were the main factors that determined the Ca concentration in the three fruit sectors (core, middle and outer cortex), as this prevalence and functionality of vascular bundles resulted in Ca enriched areas in the fruit. Ca was also present in high concentrations within the vascular bundle at 80 dafb. These enriched areas of Ca caused by the presence of vascular bundles, were less obvious in fruits analyzed at harvest. Average Ca concentrations were much higher for fruits sampled at 80 dafb compared to harvest. This is in agreement with existing literature that ascribes it to a dilution effect (Saure, 2004). Foliar Ca applications did not significantly influence the total Ca concentration of the fruit compared to the effect from enriched areas resulting from Ca delivery via active vascular bundles. However, foliar sprays did play a significant role in increasing the Ca concentrations in the outer cortex relative to the core, whereby altering the distribution of Ca in the fruit tissue. At

80dafb and at harvest, treatments where foliar sprays were applied had a higher average Ca concentration in the outer cortex relative to the core. It is therefore not surprising that previous results showed that early foliar sprays are effective in decreasing bitter pit incidence (De Villiers and Hanekom, 1977; Terblanche *et al*., 1980; Ferguson and Watkins, 1989; Saure 2002; Saure, 2005; Lötze and Theron, 2007), as it increased the Ca concentration in the areas where symptoms are typically expressed.

The efficiency of different foliar products in increasing fruit Ca was also evaluated. Current literature shows that the efficiency of a product is highly correlated to the formulation and timing of application (De Villiërs and Hanekom, 1977; Harker and Ferguson, 1991; Schlegel and Schönherr, 2002; Lotze and Theron, 2006; Joubert, 2007). When applied to product specifications, existing products (Calflo^{TM} and Calcimax^{TM}) outperformed new formulations of Calcimax^{TM} (Foliar GS^{TM} and Foliar GG^{TM}) as far as fruit Ca concentration at harvest was concerned. The application of Calflo^{TM} resulted in fruits with significantly higher Ca compared to treatments of Foliar GS^{TM} and GG^{TM}. The Calflo^{TM} treatment did not differ significantly from the Calcimax^{TM}. However, fruits from the Calflo^{TM} treatment had slightly higher Ca concentrations in spite of the similar active Ca percentage of 12%. The lower efficiency resulting from the application of Foliar GS^{TM} and Foliar GG^{TM} can be attributed partially to the fact that both have a lower active Ca percentage of 10%. The addition of adjuvant Lecithin to Calcimax^{TM} did not improve its uptake.

In the ‘Golden Delicious’ trial, the commercial spray application out-performed the other treatments. The commercial application (seven weekly applications of Calcinit^{TM}) resulted in fruit with significantly higher fruit Ca concentrations than the other treatments. However, fruit showed significantly higher starch break down percentages at harvest (more mature) and

significantly lower firmness values after storage. As determined by a bulk, fruit mineral analysis at harvest, the application of Messenger™ did not result in any significant changes in fruit Ca concentration, but showed a significant increase in fruit colour (more yellow) without affecting other quality parameters (maturity).

In both trials, the treatments that resulted in the highest amount of total Ca applied per tree, also resulted in higher fruit Ca concentrations at harvest. This effect is greater than expected, when taken into account that only a few grams of Ca (approx 11g) are applied during the season per treatment calculated on a per tree basis. This, along with timing of application, seems to be the two most important factors as far as uptake efficiency of a formulation is concerned, where later (closer to harvest) Calcinit™ applications resulted in higher fruit Ca concentrations determined at harvest.

It has been shown that the penetration efficiency of a foliar spray is much higher in the beginning of the season (Hanekom 1975; Schlegel and Schönherr, 2002; Lötze and Theron, 2006). When considering the early initiation of bitter pit (Ferguson and Watkins, 1989), together with early disintegration of xylem vessels for sensitive cultivars (Drazeta *et al*., 2004), the potential impact of these early season sprays are critical.

In the 'Braeburn' trial, satisfactorily levels of Ca, above 4.5 mg $100g^{-1}$ FW, for good fruit quality (Terblanche *et al*., 1985) were maintained by applying a series of six weekly foliar sprays from 28 dafb. Fruits also did not show signs of foliar damage due to early application of Ca or bitter pit development after storage.

To conclude, results from this study showed the importance of visual inspection to confirm active root flushes before applying Ca to the soil. This would require a bit of digging, but would ensure the correct timing and optimum uptake of soil Ca as seasonal root flushes

typically vary between localities (Eissenstat *et al*., 2006). In the 'Braeburn' trial, the application of pre-harvest soil Ca did not contribute significantly towards the Ca status of the current season's fruit and would therefore be regarded as being primarily incorporated in the tree as Ca reserves. These reserves play a significant role in supplying the developing fruit of future seasons (Terblanche *et al*., 1979; Himelrick and McDuffie, 1983). The application of pre- and post harvest soil Ca is therefore recommended for maintenance of sufficient tree Ca for initial fruit development in the beginning of the following season, when the root activity is still low. A series of early season foliar sprays is recommended to increase the Ca concentration of the present season's fruit in the outer cortex in the critical period when bitter pit initiation occurs. Although late season applications result in a higher Ca concentration in fruit at harvest, the relevancy of the Ca allocation and availability to the plant to reduce deficiencies like bitter pit is debatable.

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